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WOODLAND AVENUE AND THIRTY-SIXTH STREET, PHILADELPHIA 4, PA.

UNIDENTIFIED FACTOR(S) IN YEAST AND LIVER ESSENTIAL TO CURE OF ACHROMOTRICHIA IN DOGS ON SYNTHETIC DIETS

DOUGLAS V. FROST AND F. PEIRCE DANN
Abbott Laboratories, North Chicago, Illinois

TWO FIGURES

(Received for publication December 27, 1943)

This experiment with dogs was undertaken with two objectives in view. Frost, Moore, and Dann ('41) previously reported that factor(s) in liver besides pantothenic acid are essential to the cure and prevention of graying in rats. Dann, Moore and Frost ('42) reported later that p-aminobenzoic acid, biotin, and/or inositol were ineffective against graying in rats. The authors were particularly interested then in establishing whether graying could be produced in dogs receiving all of the available synthetic B-complex factors, including adequate pantothenic acid, and if so, whether the graying could be cured by liver and yeast. A further objective was to administer all of the B-complex vitamins except choline by injection to determine whether this form of administration would provoke a different response than oral administration.

Extensive studies by Schaeffer, McKibbin, and Elvehjem ('42a, '42b), and by McKibbin, Schaeffer, Elvehjem, and Hart ('42) indicated that classical synthetic diets based on casein and sucrose and supplemented with thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and choline, will support good blood regeneration in phlebotomized adult dogs, but produce only erratic and suboptimal growth in puppies. More recently Lambooy and Nasset ('43) reported the further inadequacy of p-aminobenzoic acid and inositol to supplement the vitamins used by the Wisconsin workers in pup nutrition. The latter workers reported complete failure of their pups at 100 to 150 days unless yeast or liver concentrate were given. The following experiments are considered in light of the above reports.

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EXPERIMENTAL

Six black puppies and one black spotted 2-month-old mongrel puppy were placed on one of the two diets shown in table 1. The pups, comprising six females and one male, were all in excellent condition and free from worms when the experiment was begun. Since there was one set of three littermates and another of two, it was possible to set up the experiment to study to advantage the effect of injection versus oral vitamin supplementation (table 2).

TABLE 1

Composition of diets and vitamin supplements.

	DIET I B-COMPLEX INJECTED	DIET II COMPLETE ORAL
Casein (S.M.A. vitamin-free)	19%	19%
Sucrose	73%	73%
Corn oil	3%	3%
Haliver oil	0.5%	0.5%
Salts U.S.P. I	4%	4%
$CuSO_4 \cdot 5H_2O$	0.004%	0.004%
$ZnSO_4 \cdot 7H_2O$	0.004%	0.004%
Choline chloride	0.67%	0.67%
l-cystine	....	0.1%
p-aminobenzoic acid	....	0.04%
Ascorbic acid	....	0.01%
i-inositol	....	0.009%
Thiamine chloride hydrochloride	0.1 mg./kg./day	0.00053%
Riboflavin	0.1 mg./kg./day	0.00053%
Nicotinamide	2.0 mg./kg./day	0.0106%
Pyridoxine hydrochloride	0.06 mg./kg./day	0.00032%
Ca-d(+)-pantothenate	0.2 mg./kg./day	0.00106%

TABLE 2

DOG [1]	ONSET OF GRAYING MONTH ON DIET	DEGREE OF GRAYING [2]	GRAYING CURED BY ADDITION OF	BLOOD VALUES Top levels before liver or yeast therapy H.B.	R.B.C.	W.B.C.	Top levels after liver or yeast therapy H.B.	R.B.C.	W.B.C.
1 ♀	2	+++	3% Liver extract [3]	14.5	4.9	10,000	17.7	8.5	10,500
2 ♀	2	+++	15% Brewer's yeast	13.8	5.4	10,300	14.6	6.5	14,650
3 ♀	11	+	Gray at death	15.1	5.5	10,000	(8.6) [4]	(3.75)	(6900)
4 ♀	8	+	Gray at death	15.7	5.9	12,050	(9.7)	(5.1)	(7900)
5 ♂	6	+++	5% Liver extract [3]	15.5	5.6	12,200	13.9	5.8	13,100
6 ♀	9	++	10% Brewer's yeast	13	4.2	6,850	16.8	6.8	14,350
7 ♀	5	+	Liver paste (1: 20) 12 gm. per day	15	5.7	6,500	17.6	7.2	12,700

[1] Dogs 1 and 2 and dogs 3, 4, and 5 were littermates.
Dogs 1, 3, 5, 6, and 7 received synthetic B-complex vitamins by injection.
Dogs 2 and 4 received all supplements orally.

[2] +++ = gray over most of body, ++ = about half gray, + = graying slight but distinct.

[3] Liver extract — fraction insoluble in 70% alcohol.

[4] Values in parentheses are blood levels about 10 days prior to death.

Two pups were fed diet II (table 1), thus receiving all nourishment by mouth. The level of calcium pantothenate chosen both for injection and as per cent of the diet allows a daily intake of about twice the requirement figure stated by Schaeffer, McKibbin, and Elvehjem ('42a) for this compound in growing pups. The level of other vitamins, including choline, was also in accord with levels used by the Wisconsin workers. The levels of cystine, p-aminobenzoic acid, ascorbic acid, and inositol in diet II were arbitrary since they are not known to be needed by the dog.

The remaining five dogs were fed diet I (table 1) and the synthetic B-complex vitamins were administered in the following manner.

A sterile, isotonic solution made to contain (per milliliter of solution) 1 mg. thiamine HCl, 1 mg. riboflavin, 35 mg. nicotinamide, 0.6 mg. pyridoxine HCl, and 2 mg. calcium pantothenate, was injected subcutaneously three times weekly in amounts calculated to supply the per kilogram body weight allowance shown in diet I (table 1). The injection solution contained 0.5% boric acid as a preservative and was sterilized by filtration. The solution was filled into 10 ml. standard rubber-stoppered vials and stored in the cold to minimize destruction of thiamine and pantothenate. Tri-weekly injection of this solution was carried out easily and with no apparent ill effect to dogs for as long as 18 months.

Although the dogs on diet I, unlike those on diet II, did not get p-aminobenzoic acid, inositol, cystine, or ascorbic acid routinely, trials of p-aminobenzoic acid and inositol were made on the injected dogs, as will be detailed later. The presence of cystine and ascorbic acid in diet II did not appear to have nutritional significance for this experiment.

Since graying appeared first in the growing dogs at the nape of the neck and base of the tail, one of these areas was shaved prior to addition of supplement to the diet. With this device, the effect of the supplement on hair growth and pigmentation was apparent sooner than it otherwise would have been. Active supplements promoted both increased hair growth and repigmentation which were readily apparent in the shaved area in 4–6 weeks. When no supplement or an inactive supplement was given, hair growth was very slow and the new hair was predominantly gray. Photographs were made to supplement these observations. The photograph in figure 1 illustrates the marked depigmentation of new hair in a shaved area on a young dog receiving diet I.

Blood studies were made by standard methods on 5 ml. samples drawn from the radial vein into a 10 ml. syringe wetted with oxalate solution.

Fig. 1 Dog 2, showing typical graying on basal diets with marked graying of new hair in the shaved area at the nape of the neck.

RESULTS

Growth of dogs 1–6 is shown in figure 2. The growth record of dog 7 was uneventful and is not shown. The littermate female pairs, dogs 1 and 2, and dogs 3 and 4, grew at equal rate as seen in the figure. Although all the dogs grew well as to stature, they did not have the sleek, plump appearance of really well-nourished dogs. Supplements of liver extracts or yeast caused improvement in the general appearance of the treated dogs, but this was not accompanied by a weight increase in every case.

Littermate dogs, 3, 4, and 5, grew rapidly to maturity (fig. 2), but declined gradually after reaching the peak of the rapid growth period. Both the activity and appetite of these dogs appeared to lessen over a period of months. When severe anorexia set in, dog 5 was placed on liver extract therapy after which activity and appetite increased. Dogs 3 and 4 continued to lose weight to the point where they refused food entirely. They were sacrificed after about 2 weeks abstinence from food and following the onset of a bronchial infection which appeared in both dogs at about the same time.

Dog 6 (injected) alone showed a "saw-tooth" weight curve during the rapid growing period. This dog appeared to show growth recoveries alternately on a total injection of 250 μg. of biotin in the form of a concentrate [1] from liver residue and a total injection of 60 mg. of p-aminobenzoic acid (fig. 2). These responses cannot be considered significant, however, since they occurred in only one of seven dogs. Furthermore,

[1] The concentrate, which contained 0.13% biotin on a dry basis, did not contain significant amounts of other known B-complex factors.

dogs which received p-aminobenzoic acid throughout showed no untoward differences from dogs which received none.

The time when graying was first seen in the different dogs ranged from 2 to 11 months. The degree of graying also varied considerably as shown in table 2. The time of appearance and the degree of graying were fairly constant among littermates and about equal for both basal diets.

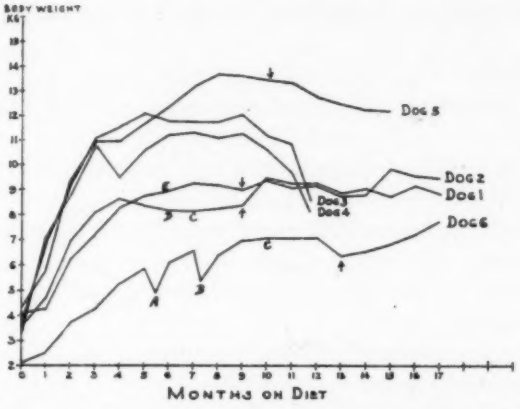

Fig. 2 Growth of dogs 1–6. Dogs 1, 3, 5, and 6 received diet I. Dogs 2 and 4 received diet II. At the points indicated by letters, the following injections were made. A 25 μg. biotin (biotin concentrate) for 10 consecutive days; B, 20 mg. of p-aminobenzoic acid for 3 consecutive days; C, 0.5 mg. p-aminobenzoic acid plus 3 mg. inositol daily for 2 months; D, 0.5 mg. p-aminobenzoic acid daily for 1 month; E, 300 μg. weekly of biotin for 3 months. At the points indicated by arrows, supplements of liver extract or yeast were added to the basal diets of dogs 1, 2, 5, and 6, as shown in table 2. Dogs 3, 4, and 5, were sacrificed at the points indicating the ends of their growth curves.

Dogs 1 and 6 received p-aminobenzoic acid (0.5 mg. per kilogram body weight) and inositol (3 mg. per kilogram body weight) daily by injection for 8 weeks during which no effect on hair growth or pigmentation were seen. Dog 2 received by injection 300 μg. of biotin per week in concentrate form for 12 weeks, also without apparent effect.

All dogs except 3 and 4 were finally fed either brewer's yeast or liver extracts as shown in table 2. Appetite response and weight gain were better with 10% than with 15% brewer's yeast. Liver extract paste (1:20)[2] fed at 12 gm. per day (dog 7) gave a marked weight response and a clearcut cure of achromotrichia. The response to 5% alcohol insoluble liver fraction[2] was more rapid than with 3% of the same

[2] Liver extract paste (1:20) represents the fraction of a hot water extract of ground fresh liver which is soluble in 70% ethyl alcohol. The alcohol insoluble fraction is that fraction of the same hot water extract which is insoluble in 70% alcohol.

fraction. All dogs showed a clearcut demarcation of new black hair in the shaved area within 6 weeks after therapy was begun, and the dull, coarse texture of the entire fur coat gave way gradually to one of good texture and lustre. The displacement of gray hair from all parts of the body required 8–12 months therapy.

Blood studies were made at 2-week intervals during the latter part of the experiment. The maximum hemoglobin, erythrocyte and leucocyte levels attained before and following liver or yeast therapy are shown in table 2.

Blood value determinations indicated a low-grade anemia and leucopenia in some of the dogs before yeast or liver therapy with a response to high average values following 4–6 weeks of therapy. Dogs 3 and 4 showed a rather severe anemia shortly before death, but this may have been aggravated both by infection and inanition. A considerable increase in venous pressure with greater ease of blood withdrawal was noted consistently following yeast or liver therapy. Injection of p-aminobenzoic acid, inositol, or biotin concentrate had no measured effect on the blood picture.

The general improvement in blood picture on liver or yeast therapy ran parallel to an improvement in appetite, weight gain, increased rate of hair growth and repigmentation. A striking outward manifestation of nutritive betterment was also seen in improved muscle tone and spirit of the dogs soon after liver or yeast therapy was begun.

None of the female dogs came into heat while on the basal diets. Dog 6, which came into heat 3 months after addition of 10% yeast to diet I, was not bred successfully.

DISCUSSION

The above investigation substantiates the previous conclusion from this laboratory that yet unknown factor(s) in yeast and liver play an essential part in pigmentation of hair. Schaeffer et al. ('42b) and Lambooy and Nasset ('43) likewise reported achromotrichia in some of their dogs receiving adequate pantothenate. In work with chicks McGinnis, Norris, and Heuser ('42) reported evidence that a factor other than pantothenate supplied by yeast is essential to normal feather pigmentation. Similar observations have been made in this laboratory.

Biotin and p-aminobenzoic acid have failed consistently to demonstrate chromotrichial activity in many trials in rats, dogs, and chicks in this laboratory. Emerson and Keresztesy ('42), and Emerson ('41) have reported the inefficacy of these compounds in rats. Martin ('42) reported that a "folic acid" concentrate prepared from yeast contains

a chromotrichia factor for sulfaguanidine poisoned rats. It should be noted in this regard that Briggs, Luckey, Elvehjem, and Hart ('43) have subdivided the "folic acid" concentrate described originally by Hutchings, Bohonos, and Peterson ('41) into three separate factors for the chick, any or all of which might conceivably be achromotrichia factors for various species.

There is much accumulated evidence in the literature (Langston et al., '38; Wilson et al., '42; O'Dell and Hogan, '43; and Waisman and Elvehjem, '43) to show that important hematopoietic factor(s) for the monkey and chick are yet to be identified in yeast and liver. This investigation indicates that such factors may also be important in the nutrition of the dog.

The level of choline used in this study provides a somewhat higher intake than that provided by Schaeffer, Elvehjem and Hart ('42b), i.e. 50 mg. per kilogram per day. The low level of choline used by Lambooy and Nasset ('43), i.e. 0.2. mg. per kilogram per day, leaves a question as to whether this might not be the cause of failure in their dogs. As pointed out by the latter workers, however, the high level of 35% casein used by them might be expected to modify the choline requirement. Both of the above groups have pointed to possible impurity of unextracted sucrose and casein as limiting factors in obtaining severe deficiencies in growing dogs.

SUMMARY

Pups fed purified diets received synthetic B-complex supplements orally or by injection throughout the experiment. The response to both types of vitamin administration appeared to be equal.

The growing dogs receiving thiamine, riboflavin, nicotinamide, pantothenate, pyridoxine, and choline developed achromotrichia and decreased hair growth in 2 to 11 months on the diet. Two of the dogs finally failed after reaching maturity on the synthetic diet. Inositol, p-aminobenzoic acid, or biotin were ineffective in changing the course of the deficiency, whereas liver fractions or whole dried yeast brought about complete cures.

General improvement in body tone and in the blood picture ran parallel to the cure of achromotrichia in the dogs which received yeast or liver.

ACKNOWLEDGMENT

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RETENTION OF THE B-VITAMINS IN RARE AND WELL-DONE BEEF [1]

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(Received for publication December 21, 1943)

The vitamin content of meat usually has been reported on the raw basis but meat is seldom eaten raw. Heat losses during cooking may be expected since thiamine is heat labile and in the presence of moisture (Frost, '43) pantothenic acid is also. Thiamine, pantothenic acid, riboflavin, and nicotinic acid are water soluble and no doubt are lost to some extent in the drippings from roasts. Rare roast beef is cooked to a lower internal temperature than well-done beef and has lower weight losses in the oven. Since these factors may affect the retention of the B-vitamins, it seemed desirable to determine the losses of thiamine, riboflavin, nicotinic acid and pantothenic acid in rare and well-done beef, roasted by a method which is not only suitable for use in the home but could be controlled carefully enough for research purposes.

EXPERIMENTAL

The roasts in this study contained two ribs each. They were the 7–8th, 9–10th, and 11–12th.[3] The roasts were cut from paired wholesale ribs from carcasses weighing approximately 400 to 500 pounds. The chine bone was not removed nor was the meat loosened from the spinal processes.

A method of roasting beef for home use has been recommended by The Committee on Preparation Factors, National Cooperative Meat Investigations ('42). It gives low weight losses in the oven as well as high palatability and appears to offer reasonably good conditions for

[1] This work was supported in part by a grant from the National Live Stock and Meat Board made through the National Research Council.

[2] Cooperative project between tthe Division of Rural Home Research and the Nutrition Laboratory, Department of Animal Husbandry.

[3] The ends of the ribs were removed beginning to cut at a point 1 inch below the ribeye at the twelfth rib and cutting parallel with the rib ends; then the individual roasts were made with the knife crowding the rear edge of the twelfth, the rear edge of the tenth, the rear edge of the eighth, and the rear edge of the sixth ribs.

retaining the B-vitamins. It was modified somewhat for use in this investigation. The roasts were removed from the refrigerator at a roast temperature of 7–10°C. and cooked on their sides on a rack in an uncovered pan without water, salt or flour and at a constant oven temperature of 150°C. until the internal temperature of the meat reached 80°C. for the well-done roasts and 60°C. for the rare roasts. The internal temperature of well-done roasts does not rise after removal from the oven but that of rare roasts does. In preliminary studies it was found impossible to predict the extent of this rise, so that a uniform maximum could be attained. Therefore, the internal temperature on removal from the oven was standardized instead. The

TABLE 1

Cooking data, moisture and ether extract.

U. S. CARCASS GRADE	NUMBER OF ROASTS		TIME IN OVEN	WEIGHT LOSSES IN OVEN		MOISTURE	ETHER EXTRACT
				Volatile [1]	Drippings [2]		
			Hours	%	%	%	%
Choice							
Raw	6	Mean	..	..	..	52.9	31.2
		Range	..	..	..	49.9–56.0	28.0–34.5
Commercial							
Raw	18	Mean	..	..	..	62.9	16.7
		Range	..	..	..	56.8–68.7	8.5–24.8
Rare	9	Mean	1.9	9.0	2.2	57.8	18.0
		Range	1.6–2.5	7.4–10.3	0.8–3.7	53.6–63.7	9.9–24.3
Well-done	9	Mean	3.2	16.6	4.5	54.3	19.9
		Range	2.5–3.9	12.8–21.7	2.8–7.3	49.2–59.7	15.1–25.6

[1] Mainly water.

[2] Includes fat, moisture, and hardened crusts.

rise after removal varied from 2–6°C. After cooking, the roasts were weighed and weight losses calculated (table 1). The rare roasts were allowed to stand at room temperature for 30 minutes or until the internal temperature began to fall, whichever was the longer. The well-done roasts were allowed to stand until the internal temperature reached 70°C.

The drippings were transferred to a graduated cylinder, the fat floated off the top by the addition of hot water, and the volume adjusted to 100 ml. Vitamin analyses were made by the methods used for the meat samples.

Meat samples for vitamin determinations were obtained from right and left roasts cut alike and used as pairs, one raw and the other cooked.

After the meat had been separated from the bone, the fat and lean together were passed through a household meat grinder twice and after thorough mixing, a portion was passed through a tissue mincer (Seevers and Shideman, '41). The resulting homogenous mince was mixed thoroughly before the samples were weighed. Details of the extraction procedure were given by McLaren et al. ('44).

Analyses of left and right raw rib roasts were made from the same carcass to test the assumption that their vitamin contents would be similar enough so that they could be used as pairs. The results show that the assumption is correct within the limits of experimental error (table 3, U. S. Choice carcass). Nicotinic acid shows more variability than do the other vitamins.

Moisture and ether extract were determined on the same sample (table 1). Approximately 2 gm. of the minced sample were weighed by difference, placed in dry ether extracted filter papers, and then in dry moisture dishes. They were dried in a vacuum oven at 95°C. for 48 hours. The dry samples then were placed in a soxhlet extraction apparatus and extracted with ether for 16 hours. Several of the meat samples could be placed in the same extraction chamber.

Calculations of retention were made as follows: Dry fat-free weight per 100 gm. of raw and cooked meat was calculated and the vitamin content expressed on the dry fat-free basis. The percentage retention was then calculated from these values. The percentage recovered in the drippings was based on the total content in the roast before cooking. Since this roast was not sampled for analysis before cooking the content before cooking was calculated by proportion from the content after cooking and the percentage retention.

THIAMINE

Methods

Thiamine was determined by two methods, that of Hennessy ('42) and that of Harris and Wang ('41). The Hennessy method was modified as suggested by McIntire et al. ('42) and Hinman.[4] Slight modifications were made in the Harris procedure. A photofluorometer was used and the fat and lean were ground together before sampling. After digestion the fat was removed with chloroform. The Harris method gave more consistent recoveries than did the Hennessy. Only the Harris values, therefore, are given in the tables.

[4] Personal communication from Winifred F. Hinman of the University of Chicago.

Content

The thiamine content of the eighteen raw rib roasts from the Commercial carcasses averaged 1.3 μg./gm. by the Harris method (table 3) and 0.8 μg./gm. by the Hennessy method. The individual Harris values were from 0.1 to 0.9 μg./gm. higher than the corresponding Hennessy values.

Similar thiamine values were found in the three raw roasts from the same carcass. Pyke ('40) found that thiamine was not evenly distributed between the muscles of the individual animal, ranging in swine from 85 I. U. per 100 gm. in the tongue to 300 in the psoas. Since it is known that the weight relationships of the individual muscles change with the cut of rib, it would seem that all of the muscles in these three beef cuts must have similar thiamine contents. This was confirmed by separate analyses of ribeye and other lean tissue from these rib cuts (table 2). The finding of no difference between the muscles in the rib of beef does not eliminate the possibility of difference between other muscles of the beef carcass.

TABLE 2

Thiamine content as related to muscle composition of raw rib roasts[1]
(Micrograms per gram)

RIBS IN ROAST	ANIMAL A.		ANIMAL B.		ANIMAL C.		ANIMAL D.		ANIMAL E.	
	Ribeye	Other lean	Ribeye	Other lean	Ribeye	Other lean	Ribeye	Other lean	Ribeye	Other lean
11–12th	2.8	2.6	2.7	2.7	2.0	1.8	2.5	2.5	2.6	2.5
9–10th	2.6	2.5	2.7	2.6	1.9	1.7	2.5	2.4	2.6	2.5
7–8th	2.6	2.5	2.7	2.5	1.9	1.9	2.5	2.5	2.5	2.6

[1] These analyses were made on lean tissue from which the fat had been removed. The values should be higher than when the fat is included in the sample.

Animal variability is indicated by the wide range in thiamine content of the raw roasts from the Commercial carcasses (table 3). Statistical treatment showed that the difference between animals was highly significant. Fatness may not influence thiamine content of beef if fat and lean are not separated since the thiamine content of the raw roasts from the Choice carcass fell within the range of values for the roasts from the Commercial carcasses (two grades below Choice). Whether this is the result of an increase in the thiamine content of lean or whether the extra fat (table 1) contained an appreciable amount of thiamine was not determined.

In the literature, thiamine values of 1.7 and 2.8 μg./gm. were reported by Waisman and Elvehjem ('41) for beef round; of 1.14 μg./gm. for top round by Booher and Hartzler ('39); and of 0.63 μg./gm. for beef

round by Cheldelin and Williams ('42). The range of 0.8 to 1.7 μg./gm. found in beef ribs in the present study is intermediate between these values for round. Since beef round contains such a large number of muscles, muscle variation cannot be excluded, but it is suggested that animal variation may account for some of the differences in thiamine content of beef reported in the literature.

Cooked roasts of beef in the present study showed the following thiamine content in micrograms per gram: by the Harris method, rare 1.2 (range 1.1–1.5), well-done 1.1 (range 0.8–1.4); by the Hennessy method, rare 0.9 (range 0.7–1.1), well-done 0.7 (range 0.5–0.9).

Retention

Retention of thiamine averaged 75% in rare roasts and 69% in well-done roasts (table 4). Retentions for the three roasts within a carcass were quite similar. The difference in retention between rare and well-done roasts was small but statistically was highly significant. Differences between rare and well-done roasts were observed in maximum internal temperature, time in oven, and weight losses in the oven (table 1), all of which may have influenced thiamine retention.

Waisman and Elvehjem ('41) reported 44 and 45% retention in two samples of roasted beef round but gave no details of their method of roasting. Since retention in their samples was considerably below those obtained in the present study, it may be assumed that their cooking conditions were less favorable for thiamine retention. Later, the Wisconsin workers (McIntire et al., '42) considered that in their previous work on retention the methods of cooking and preparation of the samples had not been properly standardized. Using a method of cooking similar to the one used in the present study, they obtained in roast pork an average retention of 70% of the thiamine. This agrees well with the average retention of 69% found here for well-done beef.

Retention in the drippings in the present study was only a small percentage of the total, but the drippings were so dark in color that the tests were not entirely satisfactory. Even adsorption failed to take out all of the color. The large amount of evaporation which occurred is indicated by the volatile losses (table 1). The roasting pan contained no added water or fat and the amount of each in the drippings was so small that tiny pointed crusts were formed where the meat juices dripped into the pan. By the end of the cooking period they were dry and hard and remained insoluble even when papain was used for digestion. Under such conditions little thiamine retention could be expected. The Wisconsin workers (McIntire et al., '42) also reported small recoveries from the drippings of pork roasts.

RIBOFLAVIN

Methods

Riboflavin was determined on papain-takadiastase extracts from which the fat had been removed with chloroform, and the proteins by precipitatation at pH 6.8–7.0. Two methods of determining the vitamin were used, a simplified fluorometric method (McLaren, et al., '44), and the microbiological procedure of Snell and Strong ('39) with modifications in the preparation of the sample to eliminate the effect of foreign growth stimulants.

TABLE 3

Vitamin content of the raw rib roast of each pair.

ROAST NUMBER	RIBS IN ROAST	MICROGRAMS PER GRAM MOIST BASIS				
		Thiamine (Harris)	Riboflavin		Nicotinic acid	Pantothenic acid
			Fluorometric	Microbiological		
		U. S. Choice Carcass				
1059 L	11–12th	1.3	1.1	1.2	33	3.1
1059 R	11–12th	1.3	1.1	1.2	32	3.0
1060 L	9–10th	1.4	1.1	1.3	29	3.2
1060 R	9–10th	1.3	1.0	1.2	32	3.1
1061 L	7–8th	1.3	1.1	1.2	28	3.1
1061 R	7–8th	1.2	1.1	1.3	34	3.1
	Mean	1.3	1.1	1.2	31	3.1
	Range	1.2–1.4	1.0–1.1	1.2–1.3	28–34	3.0–3.2
		U. S. Commercial Carcasses				
1041 R	11–12th	1.4	1.9	1.9	39	5.4
1042 R	9–10th	1.4	1.7	1.6	44	5.0
1043 R	7–8th	1.4	2.0	1.8	41	5.7
1047 L	11–12th	1.4	1.8	1.9	45	5.6
1048 L	9–10th	1.4	2.0	1.9	44	5.5
1049 L	7–8th	1.2	2.2	2.0	50	5.6
1053 L	11–12th	1.0	1.0	1.2	42	4.4
1054 L	9–10th	1.1	1.2	1.3	47	5.0
1055 L	7–8th	0.8	1.3	1.6	47	5.3
1044 L	11–12th	1.3	1.4	1.4	39	3.8
1045 L	9–10th	1.3	1.5	1.6	51	5.1
1046 L	7–8th	1.4	1.5	1.6	52	4.6
1050 R	11–12th	1.7	1.4	1.5	59	4.2
1051 R	9–10th	1.7	1.3	1.5	60	4.3
1052 R	7–8th	1.7	1.3	1.5	55	5.3
1056 L	11–12th	1.3	1.1	1.5	58	4.7
1057 L	9–10th	1.3	1.1	1.5	50	5.0
1058 L	7–8th	1.3	1.3	1.4	47	4.8
	Mean	1.3	1.5	1.6	49	4.9
	Range	0.8–1.7	1.0–2.2	1.2–2.0	39–60	3.8–5.7

TABLE 4

Retention

RARE				WELL-DONE			
Dry fat-free basis		Retained in meat [1]	Recovered in drippings [1]	Dry fat-free basis		Retained in meat [1]	Recovered in drippings [1]
Raw	Cooked			Raw	Cooked		
µg./gm.	µg./gm.	%	%	µg./gm.	µg./gm.	%	%
			Thiamine [2]				
5.99	4.77	80	3	6.79	4.76	70	..
6.26	5.00	80	2	7.04	4.62	66	..
7.00	5.51	79	2	7.07	5.07	72	..
6.96	5.22	75	1	6.54	4.72	72	2
7.19	5.31	74	3	6.62	4.66	70	1
7.28	5.19	71	1	6.30	4.28	68	1
6.44	4.74	74	2	5.65	3.81	67	2
7.00	5.03	72	2	5.71	3.54	62	1
7.13	5.23	73	2	4.13	2.96	72	2
	Mean	75	2		Mean	69	
	Range	71–80	1–3		Range	62–72	
			Riboflavin (fluorometric)				
7.08	5.89	83	..	9.99	7.54	75	..
7.89	6.11	77	..	9.66	7.24	75	..
8.14	6.51	80	..	10.71	7.86	73	..
6.59	4.93	75	..	10.10	7.53	75	..
6.88	5.52	80	..	11.27	8.28	73	..
6.57	5.38	82	..	12.96	11.28	87	..
6.24	5.61	90	..	6.57	5.44	83	..
7.51	5.85	78	..	7.19	5.65	79	..
7.34	7.33	98	..	7.65	5.87	77	..
	Mean	83			Mean	77	
	Range	75–98			Range	73–87	
			Nicotinic acid				
269.6	222.4	83	5	190.1	139.3	73	..
244.3	202.0	83	4	221.3	148.6	67	..
267.3	199.3	75	5	204.5	137.9	67	..
246.9	173.7	70	3	215.0	185.2	86	15
260.7	171.8	66	6	214.1	189.7	88	7
244.3	192.5	79	4	261.5	168.8	65	7
293.8	209.2	71	3	229.1	208.0	91	9
265.1	187.4	71	5	249.7	219.1	88	7
252.2	191.1	76	5	246.3	212.6	86	8
	Mean	75	4		Mean	79	
	Range	66–83	3–6		Range	65–91	
			Pantothenic acid				
17.5	16.3	93	4	26.3	21.0	80	..
24.3	21.9	90	2	25.0	19.3	78	..
23.7	22.4	95	3	28.5	20.3	71	..
17.7	15.7	89	2	27.0	18.5	69	3
18.4	17.1	93	3	27.0	18.6	69	4
23.2	20.8	90	2	29.3	20.5	70	2
23.4	20.0	85	1	23.7	20.3	86	3
26.3	23.4	89	1	26.5	19.9	75	1
24.7	23.5	95	1	27.9	20.6	74	1
	Mean	91	2		Mean	75	2
	Range	85–95	1–4		Range	69–86	1–4

[1] Percentage of total in roast before cooking.

[2] Percentage thiamine retained in roast beef by the Hennessy method of analysis averaged for rare, 96% (range 73–128%) and for well-done, 61% (range 50–74%).

Content

The riboflavin content of the eighteen raw rib roasts from the Commercial carcasses averaged 1.5 and 1.6 μg./gm. by the fluorometric and microbiological methods respectively (table 3).

Similar riboflavin values were obtained in the three raw roasts from the same carcass. Peterson, Brady, and Shaw ('43) have shown that the riboflavin content of pork varies with the muscle tested, ranging in the same pigs from 1.3 μg./gm. in longissimus dorsi to 3.0 μg./gm. in triceps brachii. The data in the present study indicate that all of the muscles in the rib section of beef probably have similar riboflavin values since in spite of the change in the proportions of the muscles present in the different rib cuts similar riboflavin values were obtained for them. This does not eliminate the possibility that other muscles of beef may show considerable variation.

Animal variability is indicated by the range of riboflavin values for the raw roasts from the Commercial carcasses (table 3). Statistical analyses showed the difference between animals to be highly significant. This was true for data from both methods of determining riboflavin. The riboflavin values of the raw roasts from the Choice carcass fell within the range of those from the Commercial carcasses, suggesting that fatness of the animal may have little influence on riboflavin content of beef if the fat and lean are not separated. It is not known whether this resulted from an increase in the riboflavin content of lean or whether the extra fat contained an appreciable amount of riboflavin, as was found in pork fat by Peterson et al. ('43).

Munsell ('41) reported riboflavin values for beef of 1.35 and 1.45 μg./gm. in eye muscle of rib. The average of 1.5 μg./gm. for the eighteen rib roasts from the Commercial carcasses seems to agree with Munsell's values but our values were obtained on the mixture of fat and lean and hers on the ribeye only. Waisman and Elvehjem ('41) found 1.9, 2.4, and 3.5 μg./gm. in beef round; Cheldelin and Williams ('42) 2.2 μg./gm. in beef round; and Munsell ('41) 2.14 and 2.22 μg./gm. in top round. Many of these values for round are similar to the higher ones for beef ribs in the present study.

Cooked rib roasts of beef in the present study showed the following riboflavin content in micrograms per gram: by the fluorometric method, rare 1.2 (range 1.1–1.4), well-done 1.7 (range 1.2–2.5); by the microbiological method, rare 1.4 (range 1.3–1.6), well-done 2.0 (range 1.4–2.9).

Retention

Retention of riboflavin averaged 83% in rare and 77% in well-done roasts by the flourometric method. The difference in retention between rare and well-done was small and not significant by either method. Since retentions by the two methods of analysis did not differ significantly, only the fluorometric values are given (table 4). The Wisconsin workers (McIntire et al., '42) reported retention of 85% in roast pork which agrees reasonably well with the values for roast beef obtained in the present study.

In the drippings of the present study the dark color prevented satisfactory fluorometric readings. Recovery by the microbiological method (approximately 5%) did not account for all of the riboflavin lost from the roast during cooking. The hardened crusts which remained insoluble may have contained appreciable quantities which could not be measured. The Wisconsin workers (McIntire et al., '42) recovered 5–10% in the drippings of their pork roasts which probably accounted for most of the remainder of the riboflavin in their raw meat.

NICOTINIC ACID

Method

Nicotinic acid was determined on papain-takadiastase extracts from which the fat had been removed with chloroform and the proteins by precipitation at pH 6.8–7.0. The microbiological method of Snell and Wright ('41) was used.

Content

The nicotinic acid content of the eighteen raw roasts from the Commercial carcasses averaged 49 μg./gm. (table 3). Considerable variation was observed between roasts within a carcass but since it was not statistically significant it indicates no difference in the nicotinic acid content with variation in muscle composition of the roasts. Animal variation in nicotinic acid content was shown to be highly significant by statistical treatment. That fatness may influence the nicotinic acid values is shown by the lower values obtained in the Choice carcass.

Dann and Handler ('42) reported nicotinic acid values of beef ribs to be 66, 73, and 82 μg./gm. by their chemical method. These values are somewhat higher than the ones obtained in the present study. However, they gave no data to indicate the fatness of the meat used. They reported a range of 31 to 82 μg./gm. for a large number of beef muscles.

The values 39 to 60 μg./gm. obtained in the present study fall within this wider range. They observed considerable variation between samples and attributed it to variation among individuals of the species, but suggested that some of it may have been due to the effects of ageing, over which they had no control. In the present study ageing could not be standardized but roasts 1041, 1042, and 1043 were stored the longest.

Cooked rib roasts of beef in the present study gave nicotinic acid values in micrograms per gram of 47 (range 41–53) for rare and of 46 (range 35–57) for well-done.

Retention

Retention of nicotinic acid in rare roasts averaged 75%, and in well-done roasts 79% (table 4). The difference in retention between rare and well-done roasts was small and not significant. Recovery in the drippings was too low to account for all of the nicotinic acid lost from the meat. Perhaps some of the nicotinic acid in the hardened drippings was not recovered.

The Wisconsin workers (McIntire et al., '42) obtained an average nicotinic acid retention of 85% in roast pork which agrees reasonably well with 79% in roast beef in the present study. The Wisconsin workers recovered 5–15% in the drippings which accounted for most of the remainder of the nicotinic acid in their raw meat. However, their pork drippings contained a large amount of fat and the drippings were not dry or hard. Dann and Handler ('42) reported losses during cooking of some meats but did not include roasts of beef. None of their methods of cooking was described. Perhaps loss of juices from the meats during cooking may have been sufficient to account for most of what they called surprisingly large and inexplicable losses.

PANTOTHENIC ACID

Method

Pantothenic acid was determined on papain-takadiastase extracts from which the fat had been removed with chloroform and the proteins by precipitation at pH 6.8–7.0. The microbiological procedure of Strong et al. ('41) was used with the foregoing modifications in the preparation of the sample to eliminate the effect of foreign growth stimulants.

Content

The pantothenic acid content of the eighteen raw rib roasts from the Commercial carcasses averaged 4.9 μg./gm. (table 3). There was

considerable variation between roasts in the same carcass but it was not significant. This indicates that there was little change in pantothenic acid content with variation of the individual muscles in the rib section of beef. Animal variability, however, was statistically significant. Fat may be one cause of animal variability since the values for the Choice carcass were below those for the Commercial carcasses.

Waisman and Elvehjem ('41) reported pantothenic acid values of 2.1 to 10.0 μg./gm. and Cheldelin and Williams ('42) reported 4.7 to 5.1 μg./gm. These values are for beef round but compare reasonably well with 3.8 to 5.7 μg./gm. for the beef ribs of this study.

Cooked rib roasts of beef in the present study showed pantothenic acid values in micrograms per gram of 4.8 (range 3.9–5.5) for rare and of 5.1 (range 4.6–5.6) for well-done.

Retention

Retention of pantothenic acid averaged 91% in rare roasts and 75% in well-done roasts (table 4). The difference between rare and well-done roasts was highly significant by statistical analysis. Recovery in the drippings was less than 5%. In well-done beef, it is too low to account for the remainder of the pantothenic acid.

Frost ('43) reported that while no destruction of moisture-free pantothenate occurred on heating at 120°C. for 40 hours, the presence of traces of moisture permitted slight hydrolysis at 60°C. In the presence of moisture, he found the rate of destruction to be a function of temperature. In view of Frost's findings, the internal temperature of the rare roasts may cause some destruction but the higher internal temperature of the well-done roasts should increase the destruction, and little pantothenic acid should be left in the drippings. The results of the present study, therefore, are in agreement with Frost's observations.

CONTRIBUTION OF BEEF ROASTS TO DAILY VITAMIN ALLOWANCE

The recommended daily allowances for specific nutrients set up by the Committee on Food and Nutrition of the National Research Council ('43) for a moderately active woman or a sedentary man were used as the basis for calculating the percentage supplied by one serving of roast beef. The size of the serving was the customary 4 ounces on the raw basis. When roasted by the method used here, one serving would supply approximately 7% of the thiamine, 6% of the riboflavin, and 37% of the nicotinic acid. Allowances for pantothenic acid have not been recommended.

SUMMARY

Right and left 2-rib roasts of beef were cut alike and used as pairs, one being analyzed raw and the other after cooking by a standardized method. The entire meat in each roast was ground and samples used for the determination of thiamine, riboflavin, nicotinic acid, and pantothenic acid.

The average vitamin content of the eighteen raw roasts from the Commercial carcasses was in μg./gm.: thiamine 1.3; riboflavin 1.5 and 1.6 by the fluorometric and microbiological methods, respectively; nicotinic acid 49; and pantothenic acid 4.9. Differences in vitamin content between the raw rib roasts within a carcass were not significant for any of the four vitamins but differences between animals were highly significant for thiamine, riboflavin, and nicotinic acid and significant for pantothenic acid.

Thiamine and riboflavin values for the Choice carcass were within the range of those from the Commercial carcasses (two grades below Choice) but the Choice carcass was lower than the Commercial carcasses in nicotinic acid and pantothenic acid.

Retentions in rib roasts of beef, rare and well done respectively, were: thiamine, 75 and 69%; riboflavin, 83 and 77%; nicotinic acid, 75 and 79%; pantothenic acid 91 and 75%. Retentions of thiamine and pantothenic acid were significantly lower in the well-done than in the rare roast but with riboflavin and nicotinic acid the differences between rare and well-done roasts were not significant.

One serving of rib roast of beef was calculated to furnish approximately 7% of the thiamine, 6% of the riboflavin and 37% of the nicotinic acid recommended for a moderately active woman for 1 day.

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COMPARISON OF RESPONSE OF TURKEY POULTS AND OF CHICKS TO DIFFERENT FORMS OF VITAMIN D[1]

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The vitamin D requirement of turkey poults has been reported as 60 to 70 International Units per 100 gm. of feed by Baird and Greene ('35), 200 A.O.A.C. Units per 100 gm. by Jukes and Sanford ('39) and 80 A.O.A.C. Units per 100 gm. by Hammond ('41) and by Carver and Rhian ('42). Baird and Greene ('35) used a fortified cod liver oil in determining the requirement not only of poults, but also of chicks and young pheasants. Their results with chicks would indicate the potency of this oil in A.O.A.C. Units to be the same as its potency in International Units. Jukes and Sanford ('39) used U.S.P. Reference Cod Liver Oil and also a fish oil blend, and reported that a given number of A.O.A.C. Units of the latter was 1.4 times as effective for poults as the same number of A.O.A.C. Units of the former. This would indicate a difference in the ability of chicks and poults to use different forms of vitamin D similar to the difference long known to exist between chicks and rats. Such a difference would of course help to explain differences in requirements determined in different laboratories.

Hammond ('41) found no difference between U.S.P. Reference Cod Liver Oil and a fortified cod liver oil. Carver and Rhian ('42) used a fortified cod liver oil in confirming the requirement reported by Hammond ('41). In both these investigations Activated Animal Provitamin D was also fed, but at levels which proved too high to permit accurate assessment of its potency. More recently Boucher ('44) reported that a given number of A.O.A.C. Units of vitamin D of irradiated 7-dehydrocholesterol or irradiated animal provitamin had much greater potency for turkey poults than did the same number of A.O.A.C. units of vitamin D of U.S.P. Reference Cod Liver Oil or a fortified sardine oil. The latter contained no vitamin D from any irradiation product and differed

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but slightly from Reference Cod Liver Oil with respect to the effectiveness for turkeys of a given number of A.O.A.C. Units. In the past the levels of vitamin D carriers used in turkey feeds have been universally based on the assumption that chickens and turkeys are alike in their ability to utilize different forms of vitamin D. Since irradiation products are being manufactured and used in poultry feeds in large quantities, the results reported by Boucher ('44) are of considerable practical importance, and an attempt to confirm them seemed to be warranted. It also seemed desirable to establish the potency of a commercial cod liver oil in terms of A.O.A.C. Units and test this material on poults in direct comparison with irradiation products whose efficacy for chicks had been established.

PROCEDURE

Some difficulty was encountered in obtaining poults for this work, and it was necessary to divide the investigation into two experiments. On June 23, 1943, 178 Broad Breasted Bronze poults were obtained from a commercial hatchery and divided into thirteen groups as indicated in table 1. Ten poults were started in group 1, eleven poults in groups 2 to 5, and fifteen or sixteen poults in each of the other groups.

TABLE 1

Response of turkey poults to different forms of vitamin D.
Experiment 1

GROUP	VITAMIN D SOURCE	LEVEL FED, A.O.A.C. UNITS PER 100 GM.	AVG. WT. OF POULTS IN GM. AT 4 WEEKS	EFFICIENCY TO 4 WEEKS, GM. GAIN PER GM. FEED	PER CENT OF BONE ASH AT 4 WEEKS	POTENCY OF VITAMIN D SOURCE, "POULT UNITS" PER GM. OIL
1	None	..	..	....	24.02 [1]	..
2	Reference cod liver oil, no. 2	80	289	0.448	32.65	115
3	Reference cod liver oil, no. 2	120	342	0.485	37.63	115
4	Reference cod liver oil, no. 2	160	391	0.528	39.21	115
5	Reference cod liver oil, no. 2	200	379	0.498	42.95	115
6	Cod liver oil, no. 8-117	72	278	0.442	32.53	200
7	Cod liver oil, no. 8-117	108	350	0.487	39.76	267
8	Cod liver oil, no. 8-117	144	356	0.484	40.00	204
9	Cod liver oil, no. 8-117	180	394	0.480	43.72	205
						average 219
10	Irradiated 7-dehydrocholesterol	41	227	0.376	34.99	1060
11	Irradiated 7-dehydrocholesterol	61	338	0.465	38.30	954
12	Irradiated 7-dehydrocholesterol	82	351	0.487	41.27	880
13	Irradiated 7-dehydrocholesterol	102	362	0.421	43.91	828
						average 931

[1] Bone ash at 3 weeks of age. Two surviving poults.

On June 30th, 140 poults hatched from eggs produced on the University poultry farm were divided into nine groups as indicated in table 2. Of this hatch, seventy poults were of the small White breed developed at the Beltsville Research Center and seventy were black poults, being a mixture of pure-bred Blacks, Black x White crossbreds, and White x Black crossbreds. Equal numbers of black and of white poults were placed in each group. Twelve poults were started in group 18 and sixteen poults in each of the other groups.

TABLE 2

Response of turkey poults to different forms of vitamin D.

Experiment 2

GROUP	VITAMIN D SOURCE	LEVEL FED, A.O.A.C. UNITS PER 100 GM.	AVG. WT. OF POULTS IN GM. AT 4 WEEKS	EFFICIENCY TO 4 WEEKS, GM. GAIN PER GM. FEED	PER CENT OF BONE ASH AT 4 WEEKS	POTENCY OF VITAMIN D SOURCE, "POULT UNITS" PER GM. OIL
14	Irradiated animal provitamin	48	313	0.428	36.90	760
15	Irradiated animal provitamin	72	345	.... [1]	43.57	1094
16	Irradiated animal provitamin	96	359	.... [1]	45.00	910
17	Irradiated animal provitamin	120	332	0.455	45.99	784
						average 887
18	None	..	173	0.341	30.35	..
19	Reference cod liver oil, no. 2	80	327	0.441	37.28	115
20	Reference cod liver oil, no. 2	120	331	0.455	41.29	115
21	Reference cod liver oil, no. 2	160	347	0.478	43.68	115
22	Reference cod liver oil, no. 2	200	369	0.483	44.89	115

[1] Portion of feed lost.

The basal diet used in these experiments had the following composition: ground yellow corn 29, wheat flour middlings 10, wheat bran 10, pulverized heavy oats 10, commercial acid precipitated casein 6, soybean oil meal 12, dried skimmilk 8, dried brewers' yeast 6, dehydrated alfalfa leaf meal 5, calcium carbonate 1, defluorinated superphosphate 1.5, salt 0.5, Wesson oil 2. The basal mash was analysed for calcium and phosphorus using the methods recommended by the Association of Official Agricultural Chemists ('40) for plant materials. The mash was found to contain 1.06% calcium and 0.82% phosphorus. Since the carotene content of the alfalfa leaf meal was only 55 parts per million at time of assay, beta carotene in Wesson oil was added to this diet at the rate of approximately 800 μg. per pound. The Wesson oil so added and also the vitamin D oils added replaced equivalent quantities of the Wesson oil of the formula. The cod liver oil tested for its comparative efficacy on poults and chicks in this experiment was selected as repre-

sentative of that used as a vitamin D supplement in mixed poultry feeds. The levels of the cod liver oils were calculated to furnish, respectively, 80, 120, 160, and 200 A.O.A.C. Units of vitamin D per 100 gm. of feed, and the levels of the irradiation products were calculated to furnish, respectively, 40, 60, 80, and 100 A.O.A.C. Units per 100 gm. However, the diets were made up before all the chick assay results were available. On the basis of the chick assay results, which are summarized in table 3, the number of A.O.A.C. Units of vitamin D per 100 gm. of each of the experimental diets is shown in tables 1 and 2. Chick assay number 1 was made by an independent commercial laboratory; numbers 2 and 3 were made in the laboratories of E. I. Du Pont de Nemours and Co.

TABLE 3

Assays of vitamin D sources with chicks and poults.

	COD LIVER OIL, NO. 8-117	IRRADIATED 7-DEHYDROCHOLESTEROL	IRRADIATED ANIMAL PROVITAMIN
Chick assay results in A.O.A.C. Units per gram			
Assay no. 1	157	389	468
Assay no. 2	225	425	500
Assay no. 3	160	..	..
Average	181	407	484
Poult assay results in "Poult units" per gram			
Assay no. 4	219	931	887
"Poult units"/A.O.A.C. Units	1.21	2.29	1.83

Each of the experiments with poults was of 4 weeks duration. The poults were weighed individually at weekly intervals and a record of feed consumption was kept. All poults were killed at 4 weeks of age, with the exception of group 1 which was terminated at 3 weeks, as noted in table 1. The left tibiae were removed, extracted, and ashed by groups at 850°C. according to the A.O.A.C. procedure.

RESULTS AND DISCUSSION

The average weights of the groups at 4 weeks of age, the efficiency of feed utilization, and the per cent bone ash are given in tables 1 and 2 for experiments 1 and 2, respectively. Mortality figures are not given, since mortality was negligible for all groups receiving vitamin D supplements. Excluding the basal group in experiment 1, of 168 poults started nine poults died. Excluding the basal group in experiment 2, of 128 poults started three died.

In the last column of table 1 and of table 2, the results of the bone ash determinations are interpreted in terms of "poult units" per gram of oil. For this purpose, a potency of 115 "poult units" per gram was assigned to the Reference Cod Liver Oil to correspond to its potency of 115 A.O.A.C. or U.S.P.XI Units per gram. For each experiment, per cent bone ash produced by each experimental mash containing Reference Cod Liver Oil was then plotted against the number of "poult units" of vitamin D supplied by that mash, and on the basis of the resulting points a straight line was drawn. The number of "poult units" corresponding to the per cent bone ash produced by each of the other mashes was then determined by the aid of these straight lines. The potencies thus calculated for the various levels of each of the vitamin D sources were then averaged and in table 3 these average figures are compared with the potencies in terms of A.O.A.C. chick units.

It will be noted that cod liver oil number 8-117 showed approximately the same potency whether tested with chicks or with poults, indicating that its vitamin D-active compounds were similar to those of Reference Cod Liver Oil in their comparative effectiveness for chicks and poults. This was not true of the two irradiation products. They contained approximately twice as many poult units as chick units, indicating that their vitamin D-active compounds were quite different from those of Reference Cod Liver Oil in their comparative effectiveness for chicks and poults.

These results confirm the findings of Boucher ('44), and together with those findings point to a difference between the vitamin D active compounds of the irradiation products on the one hand and those of fish oils on the other with respect to their comparative efficacies for chicks and poults. The figures presented in table 3 would seem to indicate a slight difference in this respect between the two irradiation products, but it does not appear that any significance should be attached to this difference since the biological assay method used is not very exact and since Boucher ('44) found a slight difference in the opposite direction.

The practice of evaluating vitamin D sources for turkeys on the basis of chick assays is certainly open to question, and any statement of the vitamin D requirement of poults in terms of A.O.A.C. Units requires some qualification. Considering the groups fed cod liver oil in these experiments, one would conclude that the poult's vitamin D requirement is about 200 A.O.A.C. Units per 100 gm. of feed. Considering only the groups fed the irradiation products, one would conclude that the poult's requirement is about 100 A.O.A.C. Units per 100 gm.

of feed. In neither case were sufficiently high levels fed to permit a really accurate statement of the "requirement," but the results clearly indicate the need for quotation marks around the word "requirement" when expressed in A.O.A.C. Units.

The results of these experiments do not supply an explanation of the specific disagreements existing in the literature concerning the poult's vitamin D requirement. The requirement determined in these experiments with Reference Cod Liver Oil agrees with the finding of Jukes and Sanford ('39) for Reference Cod Liver Oil and disagrees with the finding of Hammond ('41). The basal diet used by Hammond ('41) is calculated to contain 1.4% calcium and 0.8% phosphorus as compared with 1.06% calcium and 0.82% phosphorus in the diet used in these experiments. The higher calcium content and more favorable calcium: phosphorus ratio in Hammond's ('41) diet would be expected to result in a lower vitamin D requirement than would be the case with the diet used here. On the other hand the diet of Jukes and Sanford ('39) contained approximately 2% calcium and 1% phosphorus and hence should have required even less vitamin D than the diet of Hammond ('41). The diet of Carver and Rhian ('42) was very high in mineral, being calculated to contain about 2.4% calcium and 1.1% phosphorus. This fact as well as the possibility that their fortified cod liver oil contained activated animal sterol might explain the comparatively low requirement reported by them. The diet used by Baird and Greene ('35) was comparable with that of Hammond ('41) in mineral content. It contained 1.59% calcium and 0.87% phosphorus. Furthermore, the early date at which this work was done precludes the possibility that their fortified cod liver oil contained activated animal sterol.

It appears, then, that although at first glance the results of this investigation confirm the report of Jukes and Sanford ('39), these results can be reconciled with the reports of Baird and Greene ('35), Hammond ('41) and Carver and Rhian ('42) on the basis of calcium and phosphorus content of the diet whereas the report of Jukes and Sanford ('39) cannot. The use of Reference Cod Liver Oil by Jukes and Sanford ('39) and by Hammond ('41) excludes difference in vitamin D source from the list of possible explanations of the discrepancy between the reports of these investigators. Further work will no doubt be required to resolve this disagreement.

SUMMARY

Turkey poults during the first 4 weeks of life were fed a vitamin D deficient diet supplemented with four levels of each of the following

vitamin D sources: U.S.P. Reference Cod Liver Oil no. 2, another unfortified cod liver oil, irradiated 7-dehydrocholesterol, and irradiated animal provitamin. On the basis of per cent bone ash, a given number of A.O.A.C. chick units of vitamin D from irradiated 7-dehydrocholesterol and irradiated animal provitamin were, respectively, 2.29 and 1.83 times as effective for poults as the same number of A.O.A.C. chick units from Reference Cod Liver Oil. The other sample of cod liver oil was similar to the Reference Oil in relative efficacy for poults and chicks.

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SIMPLIFIED DIETS FOR THE GUINEA PIG[1,2]

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Three groups of investigators have recently reported that the guinea pig requires dietary factors which have not been isolated and identified. Hogan and Hamilton ('42) found that guinea pigs would not grow or survive long on simplified diets in the absence of crude vitamin carriers. Dried yeast and a water extract of dried liver contain the unidentified essentials. Woolley ('42, '42 a) reported evidence that guinea pigs require three or more dietary essentials in addition to those required for the growth of rats and mice. Two of these factors occurred in linseed oil meal. An adequate source of the third was not found, but its existence was postulated to account for the fact that guinea pigs die after about 4 weeks on a highly purified diet supplemented with 25% linseed oil meal plus 2 to 5% dried grass (Cerophyl). Sober, Mannering, Cannon, Elvehjem and Hart ('42) are also of the opinion that guinea pigs require three unknown dietary essentials. These factors are thought to be different from those required by the rat and the chick and are supplied by simultaneously supplementing a purified diet with yeast, dried grass and milk. Each of these supplements is presumably a good source of at least one of the three unknown factors.

Additional evidence concerning the unknown dietary essentials required by the guinea pig is presented in this paper, based upon data obtained since 1939. Preliminary experiments with approximately 600 animals served as a background for the work presented in this report. The study was planned as a progressive simplification of a satisfactory diet containing natural foods (diet G-31, a slight modification of the diet introduced by Sherman, La Mer, and Campbell ('22) for making vitamin C assays). In substituting for the natural foods, casein was used to supply protein, sucrose and rice starch to supply carbohydrates,

[1] Contribution no. 489 from the Department of Chemistry, University of Pittsburgh. The authors are indebted to Parke, Davis and Company, and to the Buhl Foundation for research grants in support of the present investigation.

[2] This paper is based upon a dissertation submitted to the Graduate School of the University of Pittsburgh by K. A. Kuiken in partial fulfillment of the requirements for the degree of Doctor of Philosophy, February, 1943.

rice cellulose to replace the crude fiber, and hydrogenated vegetable oil and corn oil to replace the fats; the salt mixture simulated the minerals of the original diet. All substitutions were based on analytical data given by Winton and Winton ('32, '37). The composition of the diets is given in table 1.

TABLE 1

Composition of diets.

CONSTITUENT	DIETS											
	G-27	G-28	G-29	G-30	G-31	G-32	G-33	G-34	G-35	G-39	G-40	G-41[1]
Wheat bran	30.0		30.0	30.0	30.0		30.0					
Rolled oats	29.0	29.0		29.0	29.0	29.0						
Skim milk powder		30.0	30.0	30.0	30.0	30.0	30.0	30.0				
Sucrose	16.5								16.4	16.4	16.4	76.0
Rice starch		18.0	21.0			18.0	21.0	39.0	38.8	38.8	38.8	
Rice cellulose		2.5	0.5			2.5	0.5	3.0	3.0	3.0	3.0	
Casein (Borden's commercial)	10.7	5.2	5.0			5.2	5.0	10.2	21.0	21.0		
Casein (vitamin-free)											21.0	18.0
Butter fat	10.0	10.0	10.0		10.0							
Hydrogenated vegetable oil		1.9	2.1	10.0		11.9	12.1	14.0	14.0	12.0	12.0	
Corn oil										2.0	2.0	1.0
Dried grass												2.0
Salts[2]	2.8	2.4	0.4			2.4	0.4	2.8	5.8	5.8	5.8	5.0
NaCl[3]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	

[1] Diet G-41 was prepared as described by Woolley ('42 a).

[2] Salt mixtures were planned to simulate the mineral content of the replaced foods. The final mixture contained 3.2 gm. $MgCO_3$, 2.3 gm. KCl, 8.4 gm. KH_2PO_4, 3.2 gm. $Na_2HPO_4 \cdot 12\ H_2O$, 0.7 gm. $Ca_3(PO_4)_2$, 1.8 gm. $CaCO_3$, 0.04 gm. K_2CO_3, 0.02 gm. NaCl, 0.06 gm. Na_2CO_3, 0.6 gm. $NaH_2PO_4 \cdot H_2O$, and 0.5 gm. $CaHPO_4 \cdot 2H_2O$.

[3] To each 100 gm. of iodized table salt were added 6 gm. iron and ammonium citrate (brown pearls), 0.2 gm. $CuSO_4 \cdot 5H_2O$, 0.26 gm. $MnSO_4 \cdot 4H_2O$ and 0.36 gm. $ZnSO_4 \cdot 7H_2O$.

Using a diet prepared in this manner it has been demonstrated that guinea pigs require an additional unknown dietary factor (or factors). When commercial casein provides the protein of the diet, small supplements of rice polish concentrate, brewers' yeast, liver extracts, dried grass, or skim milk powder permit survival and growth of guinea pigs over relatively long periods of time.

EXPERIMENTAL

Guinea pigs were purchased from a local dealer. Most of the work was done with male animals weighing from 200 to 300 gm.; both sexes were used in some of the experiments when sufficient males could not be obtained; younger animals were used during the latter part of the work. In each experiment the animals were distributed equally with regard to both weight and sex.

Individual cages with ½-inch mesh raised screen bottoms were used. Food and water were supplied ad libitum in open glass cups. All animals were weighed on alternate days. The adequacy of the diets was

TABLE 2

Oral supplements.[1]

CONSTITUENTS	SUPPLEMENTS			
	E	G	I	K[2]
Ascorbic acid, milligrams	5	5	5	5
Thiamine chloride, micrograms	50	50	50	150
Pyridoxine, micrograms	50	50	50	150
Riboflavin, micrograms	50	50	50	150
Nicotinic acid, micrograms	50	50	50	300
Inositol, micrograms	100	100	100	5,000
d-Calcium pantothenate, micrograms	200	200	200	400
Choline chloride, milligrams	5	5	5	15
Chondroitin, milligrams	5	5	5	5
Para-amino benzoic acid, milligrams		1	1	1
Calcium eriodictate, milligrams		2	2	2
β-alanine, milligrams			2	2
Pimelic acid, milligrams				1

[1] Synthetic vitamins were generously supplied by Merck and Company and Parke, Davis and Company.

[2] Animals which were fed water soluble supplement K daily, received about 1 mg. 2-methyl-1, 4-naphthoquinone weekly along with the vitamin A, D and E supplement.

judged on the basis of growth and survival over relatively long periods of time. Specific symptoms of deficiency were watched for constantly but were never observed with regularity in any one group.

The basal diets were prepared at approximately weekly intervals, and crude vitamin carriers were added not more than 24 to 48 hours before feeding. The feeding of "5%" of a supplement refers to mixing 5 gm. of the supplement and 100 gm. of the basal diet. Except in the case of diet G-41, supplements of known vitamins were not incorporated in the diet but were fed orally, by pipette. The oral supplements are listed in table 2. All animals received 5 mg. of ascorbic acid per day.

Vitamins A and D were supplied weekly at levels of 5,000 and 800 U.S.P. units, respectively, by feeding a mixture of fish liver oils [3] and vitamin D_2 [4]. In the early experiments wheat germ oil was fed by pipette to supply vitamin E; synthetic alpha-tocopherol became available at a later date and was fed weekly at a level corresponding to 1 mg. per kilo body weight per day.

Some of the diets used in this study were very powdery as first prepared and guinea pigs refused to eat them in the powdered state. For this reason, such diets were mixed with a small amount of water, kneaded thoroughly and dried overnight in a low temperature oven (maximum 55°C.). The diets thus prepared were readily eaten by guinea pigs. A control experiment with a commercial ration indicated that this process did not alter the growth promoting properties of the diet.

RESULTS

Only representative data obtained during the last 3½ years are included in tables 3 and 4. These outline the approach to the problem and provide evidence that the guinea pig requires unknown dietary factors. Diets G-27 through G-34 were used in the progressive simplification of the natural food diet. The success with these led to the formulation of G-35 free of any of the natural foods of the original diet. G-39 differs from G-35 by containing 2% corn oil in place of an equivalent amount of hydrogenated vegetable oil. An 80-day period was selected for comparing various supplements to diets G-35 and G-39, although many of the experiments were continued for more than 100 days.

Guinea pigs do not grow appreciably when fed diet G-39 and a supplement of known vitamins plus various suggested nutritional factors. However, they survive for long periods of time upon such a ration. Seven of a group of ten animals lived for over 100 days on diet G-39 plus oral supplement G. Survival and growth were improved when the following crude vitamin carriers were fed as supplements to diets G-35 or G-39: rice polish concentrate,[5] brewers' yeast,[6] powdered liver extract,[7] dried grass [8] or skim milk powder. Of these individual supplements, rice polish concentrate was the richest source of the unknown factor required under the conditions described. An average growth

[3] Natola, kindly supplied by Parke, Davis and Company.

[4] Drisdol, crystalline vitamin D_2 in propylene glycol, kindly supplied by the Winthrop Chemical Company, Inc.

[5] Labco.

[6] Pabst dried brewers' yeast.

[7] Wilson's powdered liver extract 1-20 kindly supplied by Wilson Laboratories.

[8] Generously supplied by the Cerophyl Laboratories.

rate of 2.2 gm. per day for an 80-day period was obtained when diet G-39 was supplemented with only 5% of this product.

When 10% of rice polish concentrate was fed along with 10% of commercial milk-vitamin-concentrate (diet G-35), growth was only equal to that obtained with rice polish concentrate at a 5% level. Combining

TABLE 3

Growth of guinea pigs on simplified diets.

RATION AND SUPPLEMENTS	ORAL SUPPLEMENT	NO. OF ANIMALS	TEST PERIOD	NO. OF SURVIVORS	AVERAGE GROWTH
			days		*gm./day*
G-27 + 5% M.V.C.[1]		16	100	9	3.7
G-28 + 5% M.V.C.		6	100	5	4.1
G-29 + 5% M.V.C.		6	100	6	4.0
G-30 + 5% M.V.C.		6	100	6	4.1
G-31 + 5% M.V.C.		5	100	4	4.4
G-32 + 5% M.V.C.		8	70	8	2.6
G-33 + 5% M.V.C.		8	120	6	2.7
G-34 + 5% M.V.C.		13	50	9	1.6
G-34 + 5% M.V.C. + 50 micrograms thiamine chloride per day		14	100	11[7]	2.8
G-35 + 10% M.V.C. + 10% R.P.C.[2]		14	80	11[8]	2.1
G-39	G	10	80	7[9]	0.12
G-39 + 5% M.V.C.	E	14	80	8[10]	1.0
G-39 + 5% R.P.C.	E	19	80	17[11]	2.2
G-39 + 5% R.P.C.	G	10	80	9	2.1
G-39 + 8% liver[3]	G	5	50	4	2.1
G-39 + 6% grass[4]	G	5	80	2	1.5
G-39 + 5% yeast[5]	G	11	80	9	1.4
G-39 + 5% S.M.P.[6]	G	11	80	9	2.2
G-39 + 5% R.P.C. + 8% liver	G	5	50	5	1.8
G-39 + 5% R.P.C. + 6% grass	G	5	80	5	3.0
G-39 + 5% R.P.C. + 5% yeast	G	11	80	10	2.6
G-39 + 5% S.M.P. + 5% yeast	G	8	80	8	2.7
G-39 + 5% R.P.C. + 5% S.M.P.	G	12	80	10	3.4

[1] M.V.C. = 70% ethanol extract of Borden's milk vitamin concentrate; 1 ml. = 1 gm. original product.

[2] R.P.C. = Labco Rice Polish Concentrate.

[3] Powdered liver extract 1-20 from the Wilson Laboratories.

[4] Dried grass from the Cerophyl Laboratories.

[5] Pabst brewers' yeast.

[6] S.M.P = skim milk powder.

[7] Eleven animals continued successfully for 210 days.

[8] Seven animals continued successfully for 180 days.

[9] Died after 110 to 150 days on experiment.

[10] One lived 250 days; all others died during the first 120 days.

[11] Nine of an original group of 12 were continued and grew at an average rate of 1.6 gm. per day for a 190 day period.

rice polish concentrate with liver or yeast did not greatly improve the results obtained with rice polish alone, but further growth stimulation was observed when rice polish concentrate was combined with either skim milk powder or dried grass. An average growth rate of 3.4 gm. per day was obtained by supplementing diet G-39 with 5% of rice polish concentrate plus 5% of skim milk powder and oral supplement G.

Representative results obtained with basal diets of higher purity (G-40 and G-41) are presented in table 4. Diet G-40 was prepared by replacing the commercial casein of diet G-39 with "vitamin-free"

TABLE 4

Experiments with diets containing vitamin-free casein.

RATION AND SUPPLEMENTS [1]	NO. OF ANIMALS	NO. OF SURVIVORS			AVERAGE GROWTH (AT 20 DAYS)
		20 days	30 days	40 days	
					gm./day
G-40 (S.M.A. casein) + oral supplement G	20	10	3	2	— 3.1
G-40 (Labco casein) + oral supplement G	20	10	3	2	— 2.6
G-40 + 10% S.M.P. + oral supplement G	10	8	4	4	— 2.5
G-40 + 10% R.P.C. + oral supplement G	10	7	2	0	— 2.6
G-40 + 5% R.P.C. + 5% S.M.P. + oral supplement G	10	5	4	2	— 1.0
G-40 + 5% R.P.C. + 10% grass + 1% glycine + oral supplement I	6 [2]	5	4	0	0.0
G-40 + 5% R.P.C. + 5% S.M.P. + 10% grass + 1% glycine + oral supplement I	7 [2]	7	6	0	0.10
G-40 + 10% liver + 10% grass + oral supplement I	11 [2]	4	0	0	
G-40 + 10% yeast + 10% grass + oral supplement K	34 [2]	16	6	5	0.52
G-41 (Basal controls)	10 [2]	3	2	1	0.17
G-41 + oral supplement K	10 [2]	6	4	3	0.50
G-41 + 25% linseed oil meal	10 [2]	6	2	2	0.50
G-41 + 10% R.P.C.	10 [2]	10	8	6	1.4 [3]
G-41 + 10% S.M.P.	10 [2]	7	6	4	0.07
G-41 + 10% yeast	10 [2]	8	8	7	1.3 [4]
G-41 + 10% liver	10 [2]	10	10	10	0.75 [5]
G-41 + 10% R.P.C. + 10% S.M.P.	10 [2]	10	9	8	1.0 [6]
G-41 + 10% R.P.C. + 10% M.V.C. + oral supplement K	10 [2]	10	7	4	1.2

[1] Refer to table 3 for explanation of abbreviations and identification of supplements. Unless otherwise indicated, Labco Vitamin-free casein was used in preparation of the diets.

[2] Starting weights ranged from 125 to 200 gm. All others ranged from 200 to 300 gm.

[3] Survivors averaged 1.4 gm./day for 40 days.

[4] Survivors averaged 0.36 gm./day for 40 days.

[5] Survivors averaged 0.64 gm./day for 40 days.

[6] Survivors averaged 1.0 gm./day for 40 days.

casein. Guinea pigs did not survive on diet G-40 in contrast with their performance on diet G-39. The crude vitamin carriers which were effective in earlier experiments only permitted slight improvement in the survival and growth of guinea pigs in comparison with their performance on the basal ration alone. The crude vitamin carriers which were markedly effective in earlier experiments only produced slight improvement in the survival and growth of guinea pigs in comparison with their performance on the basal ration alone. The commercial casein (21%) of diet G-39, therefore, either supplied a basal level of some necessary factor which was increased to more nearly an adequate level by the various supplements or it carried an essential material different from those provided in appreciable dosage in the supplements. If only one essential were involved, the large additions to the vitamin-free casein diet should at least have permitted survival comparable with that on the unsupplemented commercial casein diet. Of the 129 animals on diet G-40 plus supplements (of 10% at least, and half the group received a 20% supplement), 91 animals survived 20 days, only 12 survived 40 days and none lived beyond 50 days. In contrast, on diet G-39 without any supplement, 7 of a group of 10 survived 110 days or longer, and on G-39 plus a 5 or 10% supplement, 96 of 116 animals survived 80 days (end of experimental period) and grew fairly satisfactorily. These data may not exclude the possibility of explaining the differences in performance on the basis of different levels of the same factor, but they argue very strongly for two different factors — one supplied by the commercial casein and another by the various supplements. The authors are fully convinced that the data justify the latter interpretation.

Diet G-41, used successfully by Woolley ('42, '42 a) in assays with very young animals, also was used to test the above group of crude vitamin supplements. Guinea pigs ate this diet more readily from the start than diet G-40 and, accordingly, growth stimulation over a short period of time could be more easily demonstrated. Rice polish concentrate increased growth more uniformly than other supplements. Liver extract was very effective in extending the average survival time but did not have an equal effect on growth. The one sample of linseed oil meal tested did not markedly improve either growth or survival. However, the results should not be compared directly with Woolley's observations because our animals were not as young as those he used. A complete diet was not produced in any of the combinations, in the sense of maintaining *normal* growth. Survival of guinea pigs on the highly purified diet was barely extended beyond 40 days.

DISCUSSION

Guinea pigs survived and grew although at a restricted rate, for long periods of time on simplified diets containing not more than 5% of crude vitamin concentrates. One group of nine animals was discontinued after 190 days with an average growth of 1.6 gm. per day. The successful diets can hardly be referred to as "purified diets", however, because they did not consist solely of highly purified ingredients. When an attempt was made to use "vitamin-free" casein in the preparation of the diet, guinea pigs failed to grow or survive satisfactorily. Commercial casein (Borden), therefore, contains a generous supply of a dietary factor essential for the guinea pig, beyond those required by rats, that is not present in "vitamin-free" casein.

It was not possible to characterize the limiting factors involved when the study was extended with diets containing purified casein. Although biotin and "folic acid" were not available for testing, it is doubtful whether a deficiency of either of these factors was involved. Cow's milk has been reported to be extremely low in "folic acid" (Cheldelin and Williams, '42), and casein or skim milk powder therefore, would not be expected to carry it in large enough quantities to account for the results. On the other hand, milk is a good source of biotin (Cheldelin and Williams, '42; and György, '39) and the change in casein might have critically reduced the intake of this factor. However, yeast and fresh liver have been reported to be good sources of biotin (György, '39), and it will be noted that growth stimulation was not observed when 10% of either dried brewers' yeast or liver extract was added to diet G-40 plus 10% of grass. Since no direct analytical data are available on the yeast and liver preparations tested, though they were standard high quality products, it can only be assumed that they were contributing significant amounts of biotin. The rice polish concentrate used in this work contained only 1.4 μg.[9] of biotin per gm. of solids and, therefore, cannot be regarded as a very potent source of this factor. This indirect evidence suggests that neither biotin nor "folic acid" is the factor supplied by commercial casein.

Sober, Mannering, Cannon, Elvehjem and Hart ('42) have reported that the general condition of their guinea pigs was improved when the amount of casein in the basal ration was raised from 18% to 30%. Since the casein used in their ration was "relatively unpurified" the beneficial effect observed may be related to an increased supply of the unknown dietary essential reported here. Our data suggest that this factor precipitates with the casein fraction during the commercial

[9] Data kindly supplied by G. C. Supplee of Borden Company Research Division.

preparation of casein, since a 10% skim milk powder supplement did not permit a detectable growth response in animals receiving a "vitamin-free" casein diet even though it may have improved the survival slightly.

Animals showed a relatively good survival when fed the basal diet (containing commercial casein plus oral supplement G), but they failed to grow satisfactorily. Only a few crude vitamin carriers were tested as supplements to this diet. Rice polish concentrate was consistently found to improve the diet and is regarded as the best source (of those studied) of at least one unknown factor required by the guinea pig. Brewers' yeast, powdered liver extract, skim milk powder, and dried grass were also found to be valuable as supplements, and it is thought that they contain lesser amounts of the same factor (or factors) supplied by the rice polish concentrate.

The beneficial effect of the commercial casein and also of the skim milk powder is in accord with the good growth response obtained by Sober, Mannering, Cannon, Elvehjem, and Hart ('42) to supplements of 20 ml. of milk per day fed to guinea pigs on a simplified ration. However, in contrast with the diets used in our experiments, their diets contained both yeast and dried grass, and their supplements of known vitamins were more limited both qualitatively and quantitatively. Our oral supplements (table 2) included not only liberal quantities of known vitamins, but also a number of materials which have been suggested as essential nutrients. The necessary factors supplied by the yeast and grass may have been included in part, in the oral supplements fed in the present experiments. The liberal provision of our vitamin supplement is thought to furnish good evidence that the factors reported here are different from any of the well characterized nutritional essentials. The chance that an amino acid deficiency might be involved seems very remote because of the very limited responses obtainable from the milk, yeast and dried grass supplement.

It is of interest to note that albino rats, initial weight 50 gm., grew well on diet G-39 plus 5% of the rice polish concentrate and supplement E (at one-half the guinea pig level) through a period of 50 days (average gain in body weight, 3.6 gm. per day).

"Wrist stiffness" in the guinea pig as reported by Wulzen and Bahrs ('41) and Wulzen ('42) was not observed; only nine cases of stomach ulceration (Kohler, Randle, Elvehjem and Hart, '39) were found and these were not observed to be associated with any specific variant in the diets. It must be concluded that if these are specific deficiency symptoms, the corrective factors were present in the diets studied either directly or by intestinal synthesis.

SUMMARY

1. Growth and survival of guinea pigs for long periods of time on simplified diets containing not more than 5% of crude vitamin concentrates are reported.

2. The basal simplified diet was developed through systematic replacement of the natural foods in a satisfactory ration. With equivalent amounts of protein, carbohydrate, fat, and minerals, the diet was not adequate when supplemented with known vitamins.

3. The authors interpret the data presented as indicating that at least two unknown dietary essentials are required to complete the diet of the guinea pig. When commercial casein provides the protein of the diet, small supplements of rice polish concentrate, brewers' yeast, liver extract, skim milk powder or dried grass markedly improve both survival and growth, thus demonstrating the presence of one of these essentials. However, if "vitamin-free" casein is used, these supplements, even in large amounts, have very little, if any, beneficial effect upon growth or survival, providing evidence for another factor in commercial casein.

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STUDIES ON THE ABSORPTION OF CAROTENE[1]

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ONE FIGURE

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Because of the increasing importance of the provitamin, carotene, as a source of vitamin A in the diet, it is desirable to have information on the factors which influence the absorption and utilization of this pigment from the gastrointestinal tract. There is considerable evidence that the bile plays an essential rôle in the absorption of carotene. Greaves and Schmidt ('35) proved that the bile salts such as glycodesoxycholic or desoxycholic acid were required for the absorption of this provitamin in vitamin A deficient, choledochocolonostomized rats as judged by the vaginal smear technique. Vitamin A on the other hand could be readily absorbed by such bile fistula rats. Similar convincing results on dogs have been reported by Irvin, Kopala and Johnston ('41) making use of intestinal loops; insignificant amounts of carotene were absorbed when introduced into the loops in cotton-seed oil solutions; however, when either gall-bladder bile (hog or ox) or pancreatic lipase was administered, significant quantities of carotene were absorbed. When both lipase and bile were introduced together with the carotene solution, much larger amounts were utilized. Sodium desoxycholate was considerably more effective than the sodium salts of cholic or glycocholic acid and somewhat more potent than whole bile. The differences in absorption of carotene and vitamin A have been ascribed to the fact that the latter is an alcohol while the former is only hydrocarbon.

Carotene absorption is apparently augmented when fat is also being absorbed. Wilson, Das Gupta and Ahmad ('37) found practically complete absorption of the carotene of raw or cooked carrots and of spinach when the diet contained fat while only 50% was absorbed on a fat-free diet. Van Eekelen and Pannevis ('38) obtained similar results with human subjects while Kemmerer and Fraps ('38) found in rats

[1] This was aided by a grant from The Best Foods, Inc.

increased absorption of carotene when fed in Wesson oil. Russell, Taylor, Walker and Polskin ('42) report that the absorption of carotene but not of vitamin A was decreased in hens on a fat-free ration. The percentage absorption of carotene was also higher when fed in lower concentrations. It is also possible that the absorption of this pigment may be different when fed in the pure state than when fed as a component of foods.

The absorption of vitamin A by normal rats has been shown by Reifman, Hallman and Deuel ('43) to vary with concentration. Although the rate of absorption of vitamin A was only approximately 6 I.U. per 100 sq. cm. per hour when administered in fat in a concentration of 100 I.U. per gram, it was increased to over 10,000 I.U. when the concentrate contained approximately 1,000,000 I.U. per gram.

The present study was designed to determine the rate of absorption of carotene as influenced by concentration. It has been possible not only to determine the period necessary for absorption from the lumen of the gut but also to follow the transfer from the gut wall to the tissues. Experiments are now in progress to determine when factors may influence each of these steps.

EXPERIMENTAL

Carotene dissolved or suspended in cotton-seed oil was fed by stomach tube to rats previously fasted for 48 hours and the amount of carotene remaining in the gut determined after various periods. It was necessary to place the rats which had been on the stock diet high in alfalfa, on a carotene-low diet [2] for a week prior to the fasting period in order to decrease the correction for pigment in the intestine.

Carotene was determined by the procedure of Koehn and Sherman ('40) with the Klett-Summerson photoelectric colorimeter and a filter with a maximum transmission at 420 mμ. Total fat was determined on the gastro-intestinal contents as described previously (Deuel, Hallman, and Leonard, '40). In the experiments reported in series I, carotene was determined only on the lumen contents. In the second series of tests, after removal of the contents of the lumen by flushing with 100 ml. of freshly distilled diethyl ether, the carotene was also determined in the gut wall by extraction of the gut homogenized in the Waring blendor three times with diethyl ether. The extracts from the lumen contents were dried with anhydrous Na_2SO_4, the extract filtered and made to volume, the Na_2SO_4 being thoroughly washed with ether. Caro-

[2] Similar to U.S.P. XII vitamin A depletion diet except that unextracted casein was used.

tene was determined on this sample and the total lipid was estimated on an aliquot by evaporation of the diethyl ether.

With the extracts of the intestinal wall, the extract was filtered, made to volume with diethyl ether, and the value of carotene read directly on the colorimeter. Carotene was administered in amounts of approximately 350 and 3750 μg. per ml. of fat. In the former, all the carotene was in solution; in the higher concentration, 35.2% or 1322 μg. was in solution and 64.8% or 2428 μg. was in suspension and could be separated by centrifugation at 10,000 r.p.m. for 1 hour. The mixtures were fed in amounts of 300 mg. per 100 sq. cm. of surface area. Surface area was computed by the formula of Lee ('29) based on the weight after fasting. The carotene was the S.M.A. product containing 90% beta and 10% alpha carotene.

TABLE 1

The carotene and lipid recovered from the gastro-intestinal contents of male rats previously fasted for 2 days (control tests) or immediately after the administration of carotene in cottonseed oil (recovery tests).

NO. OF TESTS	AVERAGE WEIGHT	LIPID				CAROTENE				
		Fed	Recovered			Fed	Recovered			In gut wall
			Total	Corrected	Per cent		Total	Corrected	Per cent	
Control tests		mg.	mg.	mg.		μg.	μg.	μg.		μg.
7	253	0.0	14.4			0.0	9			
3	218	0.0	13.9			0.0	1			
6	256	0.0				0.0	6			69.9
Recovery tests										
7	274	1001	1002	988	98.9	363	359	350	95.4	
5	222	888	873	859	97.0	3693	3572		96.5	

RESULTS

Table 1 records the control values of carotene and lipid in the intestinal contents and intestinal wall of fasted rats, values which are used as correction factors as well as in the estimation of the recovery of these constituents when the gut was removed and flushed with ether immediately after the carotene solutions were introduced.

Table 2 gives the summary of the average absorption at 3 hours when the carotene was fed at different concentrations (series I) and for the larger doses at various intervals for 42 hours (series II). The total recovered is the sum of that found in the lumen and gastro-intestinal wall. The total transferred refers to the quantity which is unaccounted for, presumably because it was passed through the intestinal mucosa

TABLE 2

The absorption of lipid and carotene by rats fed 300 mg. per 100 sq. cm. of carotene solutions in cottonseed oil and killed at various periods thereafter.

LENGTH OF PERIOD	BODY WT.	LIPID			CAROTENE								
		Fed	Absorbed [2]		Fed	Recovered [2]			Transferred [2]		Absorbed [2]		
			Total	Per 100 sq. cm./hour		Total	Lumen	Wall	Total	Per 100 sq. cm./hour	Total	Per 100 sq. cm./hour	Correlation coefficient [3]
Series I													
hr.	*gm.*	*mg.*	*mg.*	*mg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	
3 [1]	257	963	348±27 (144—535)	31.0±2.4 (15.7—49.1)	347		253±9 (195—316)				95±10 (26—163)	9.1±0.8 (2.5—14.7)	0.34
3	236	914	325±24 (218—449)	32.9±1.6 (31.4—45.6)	3806		2734±95 (1800—3500)				1072±130 (314—1764)	108.4±10.7 (33.8—189.5)	0.79
Series II													
3	242	931	347±24 (217—495)	34.2±2.5 (20.9—47.6)	3821	3392	2681±110 (2237—3286)	711±97 (270—1208)	429±115 (74—1289)	52.6±11.2 (4.8—128.7)	1140±108 (606—1675)	112.0±10.4 (58.5—160.5)	0.70
6	231	904	632±33 (469—872)	32.4±1.9 (22.6—42.5)	3689	2787	1912±235 (957—3025)	875±136 (305—1750)	902±205 (11—2064)	48.0±9.2 (0.6—106.7)	1774±222 (651—2611)	90.7±11.3 (33.7—137.5)	0.26
12	225	890	858	22.2	3641	2521	481±111 (80—1200)	2040±269 (260—3250)	1120±244 (499—3244)	29.1±8.1 (12.0—84.3)	3110±130 (2618—3940)	85.3±4.0 (64.3—113.5)	
18	231	906	894	15.1	3698	2140	362±135 (87—1565)	1778±226 (558—3070)	1558±218 (401—2699)	26.3±3.6 (7.1—43.7)	3330±182 (1973—4017)	56.1±2.4 (34.8—61.2)	
42	222	880	880		3805	627	49±25 (0—232)	578±176 (0—1870)	2978±228 (1775—3647)	22.1±1.4 (12.3—26.8)	3567±33 (3378—3753)		

Male rats were used in series I, female rats in series II.

[1] 14 experiments in this group only: 10 experiments in the other groups.

[2] Including the standard error of the mean calculated as follows: S.E.M. $\sqrt{\frac{\Sigma d^2}{n}}/\sqrt{n}$ where "d" is the deviation and "n" is the number of observations. The values in parentheses indicate the maximum and minimum in each group.

[3] The "rank order" coefficient shows the direct relation between rate of lipid and carotene absorption. A perfect correlation would give a value of 1.00, while a value of 0.0 would indicate no correlation. The following formula was used for its calculation $\rho = 1 - \frac{6\ \Sigma d^2}{n(n^2-1)}$ where "d" is the difference between the rank order and "n" is the number of determinations.

into the circulation. The quantity absorbed refers only to the amount which has disappeared from the lumen. This is the basis on which the absorption of fats and vitamin A has been computed in our previous work.

A comparison of the rate of absorption of both carotene and vitamin A with the concentration fed is given in figure 1. When this is based on I.U., the values for carotene at the two levels fed fall on the vitamin curve.

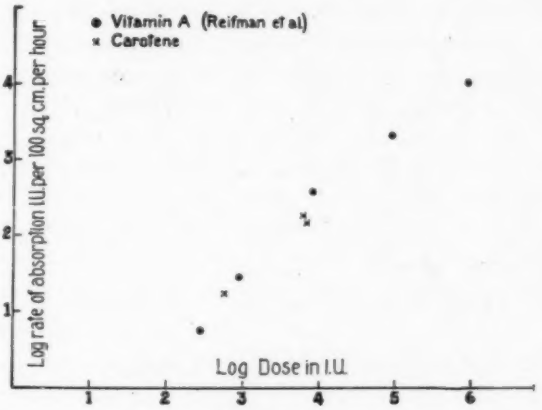

Figure 1

DISCUSSION

With a ten-fold increase in the concentration of the carotene fed, a proportionate increase in the quantity of carotene absorbed resulted. Thus, when approximately 350 μg. of carotene were administered, the average absorption was 9 μg. per 100 sq. cm. per hour. When the carotene administered was increased to 3800 μg., the average rate of absorption in the two series of 3-hour tests was 108 and 112 μg. The absorption rate dropped as the concentration in the lumen became lower, the calculated values being 91, 85 and 56 in the 6,- 12,- and 18-hour tests, respectively. When the absorption levels in the 3-hour tests are compared with the values obtained earlier for vitamin A by Reifman, Hallman, and Deuel, '43, (fig. 1), the rates of absorption correspond closely to those obtained when the similar concentrations of vitamin A were given. This is especially remarkable in view of the fact that over 60% of the carotene in the higher dose was merely suspended in the oil.

As has been indicated by a number of investigators, there is a distinct correlation between the rate of carotene and lipid absorptions. This

[3] The "rank order" coefficient shows the direct relation between rate of lipid and carotene absorption. A perfect correlation would give a value of 1.00, while a value of 0.0 would indicate no correlation. The following formula was used for its calculation $\rho = 1 - \frac{6\,\Sigma d^2}{n(n^2-1)}$ where "d" is the difference between the rank order and "n" is the number of determinations.

interrelationship was found only in the 3- and 6-hour groups; no relationship could be expected in the 12-hour group since in most cases all of the lipid had been completely absorbed.

Carotene absorption was more rapid and complete than was to be expected; less than 2% remained in the gut 42 hours after feeding. The completeness of the absorption is difficult to understand when one considers that values of only 50% were obtained by Kemmerer and Fraps ('38) when carotene was fed in Wesson oil. Although we did not analyze the feces, when any excretion occurred it was usually in the early part of the experiment before the fat had reached the large intestine. All experiments in which diarrhea occurred were discarded. The discrepancy between the complete absorption in our tests and the less complete absorption obtained by other investigators may be that in the present tests the rats were fasted while in other tests the animals were fed simultaneously with the absorption experiment. It is possible that the presence of indigestible food residues in the latter cases may have resulted in the removal of undigested carotene from the intestine before absorption could be completed since the rate of absorption is quite slow.

The accumulation of carotene in the gut wall reached a maximum in the 12-hour group at which time the intestine grossly appeared colored deeply yellow. Since the rate of disappearance for the first 12 hours is less than the rate of accumulation in the gut wall, it is evident that the rate of transfer out of the gut wall may be a controlling factor in absorption of this pigment. The maximum average rate of removal from the lumen of 112 μg. per 100 sq. cm. involves an absorption of 1150 μg. or 1920 I.U. of carotene in a 3-hour period.

The rate of absorption of the cottonseed oil was quite uniform in the 3- and 6-hour tests (31.0 and 34.2 mg. per 100 sq. cm. per hour) which is somewhat lower than the value 43.3 obtained on female rats or the values 39.7–47.9 obtained on male rats reported by Deuel, Hallman, and Leonard, '40.

SUMMARY

The rate of absorption of carotene is proportional to the dose fed. When fed in cottonseed oil at a level of 350 μg. per gram, 9 μg. were absorbed per 100 sq. cm. per hour; at a level of 3750 μg., 110 μg. were absorbed per hour. These values calculated as I.U. are practically identical with those reported earlier for vitamin A. This occurred in spite of the fact that about two-thirds of the carotene fed in the higher dose was in suspension.

The concentration reaches a maximum in the gut wall at 12 hours; considerable amounts still remained in the gut wall after 42 hours, but all had been removed from the lumen of the gut. It is suggested that in the absorption of carotene the transfer through the intestinal wall is the limiting factor since it proceeds more slowly than absorption from the lumen. There is considerable correlation between the amounts of fat and carotene absorbed.

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EFFICACY OF VITAMIN D FROM DIFFERENT SOURCES FOR TURKEYS[1]

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TWO FIGURES

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It is well known that turkeys have a higher requirement for certain specific nutrients than chickens and this is particularly true of vitamin D as first pointed out by Scott, Hughes and Loy ('32). Since then, however, there has been considerable disagreement among those who have attempted to measure the amount of vitamin D required by young growing turkeys. Baird and Greene ('35) using a single vitamin D supplement, a fortified cod liver oil, determined the levels required to prevent rachitic symptoms in growing chicks, poults and pheasants. They reported that turkeys require a minimum of 60 to 70 U.S.P. units of vitamin D per 100 gm. of feed during the first 12 weeks of life. A careful study of this work indicated that these authors may not have obtained the maximum response in calcification and growth from even the highest level of vitamin D fed in their turkey experiments and that, therefore, the requirement for maximum response could have been distinctly higher. Furthermore, the potency of their supplement was originally established in Steenbock rat units and the question of the conversion factor used in calculating U.S.P. units would also tend to raise doubt as to the true value of the units fed by them as compared with A.O.A.C. chick units used by later workers.

Jukes and Sanford ('39) were the first to attempt the quantitative determination of the response of young turkeys on a rickets-producing diet to a standard vitamin D substance, namely, U.S.P. reference cod liver oil no. 1, the potency of which was definitely known in terms of A.O.A.C. chick units. They also fed in the same experiments a sardine oil, fortified with fish liver oils, which was assayed with chicks against

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the reference cod liver oil. They found that levels of the reference cod liver oil up to and including 120 A.O.A.C. units per 100 gm. of diet produced unsatisfactory growth and calcification to 4 weeks of age. Unfortunately, they did not feed higher levels but extrapolation of their curve of response indicated that more than 200 units would be needed for maximum calcification. Surprisingly, they noted that levels of the fortified sardine oil, equivalent to the reference oil in A.O.A.C. chick units, produced a greater calcifying effect in the turkeys. This led them to suggest the possibility of a difference between chicks and turkeys in their response to different sources of vitamin D and they properly pointed out the difficulty of expressing the requirements of turkeys in terms of chick units.

Hammond ('41) in a brief paper reported the feeding of reference cod liver oil, a fortified cod liver oil and activated animal provitamin D to poults during the first 4 weeks of life as supplements to a rachitogenic diet. In sharp contrast with the findings of Jukes and Sanford ('39) he found that 80 A.O.A.C. chick units from the reference cod liver oil produced a maximum effect on body weight and bone calcification. His data on the other two supplements are of less value in estimating the efficacy of each since he relied on the manufacturer's claims for the A.O.A.C. chick unit potency. In addition, he presented no information on the source of the vitamin D used to fortify the cod liver oil, and the levels fed of activated animal provitamin D, except one, were unreasonably high. It is interesting to note, however, that the 50-unit level of activated animal provitamin D gave a response approximately equal to 80 units of the other two supplements.

After the work here reported had been started, the publication of Carver and Rhian ('42) appeared. These workers fed a fortified cod liver oil at 8 levels (50 to 200 units) and activated animal provitamin D at 1 level (100 units) to duplicate groups of poults receiving a rachitogenic diet. Both supplements were assayed by the authors and the dosage was expressed in A.O.A.C. chick units. No reference cod liver oil was fed. Carver and Rhian concluded that 80 A.O.A.C. chick units per 100 gm. of feed from either of the supplements produced satisfactory growth and calcification in young turkeys. No information was given as to the source or composition of the fortified oil.

The primary object of the experiments here described was to compare the efficacy of vitamin D from different sources for turkeys, particularly when fed on the basis of equal potency, measured in A.O.A.C. chick units. It appeared also that the observations of Jukes and Sanford ('39) were worthy of further investigation and that additional

work of this nature might help resolve the lack of agreement as to the requirement of young turkeys for this essential nutrient.

EXPERIMENTAL

The percentage composition and analysis of the basal diet are given in table 1. This diet was formulated with the idea of supplying optimum amounts of all the essential nutrients with the exception of vitamin D. It resembled typical turkey starting mashes except that casein was used in place of meat scraps or fish meal in order to avoid possible contamination with vitamin D from animal sources. The dehydrated alfalfa meal was of high quality as shown by analyses indicating a carotene content equivalent to 92,400 U.S.P. units of vitamin A per pound

TABLE 1

Percentage composition and analysis of basal diet.

Ground yellow corn	25.9	Ground oats	10.0	Dried brewers' yeast	6.0
Soybean oil meal	12.0	Dry skimmilk	8.0	Ground limestone	2.0
Wheat bran	10.0	Dehydrated alfalfa meal	7.5	Corn oil Mazola)	2.0
Wheat middlings	10.0	Commercial casein	6.0	Sodium chloride	0.5
				Manganese sulfate	0.1

Protein (N x 6.25) %	24.56	Crude fiber %	5.18
Moisture %	9.74	Calcium %	1.05
Ash %	5.46	Phosphorus %	0.63
Ether extract %	5.43	Manganese %	0.01

Riboflavin — micrograms per 100 gm.	575
Vitamin A — International units per 100 gm.	1724

and a riboflavin content of 5,630 μg. per pound. A vitamin D assay of the alfalfa meal using rats as experimental animals showed that it contained between 363 and 454 U.S.P. units per pound. This amount was considered negligible since it was presumed to be the ineffective activated plant sterol type. The results proved this to be true.

A preliminary trial employing 3 groups of poults was conducted for the purpose of testing the suitability of the basal diet described in table 1 and of securing some indication of the response which might be obtained to two supplements representing diverse sources of vitamin D. One group of poults received the basal diet alone, the second received as a supplement to the basal 115 A.O.A.C. units per 100 gm. of diet from fortified sardine oil, while the third received as a supplement 105 A.O.A.C. units per 100 gm. of diet from irradiated 7-dehydrocholesterol. The groups were continued for 4 weeks on these diets. On the twenty-eighth day the survivors were killed and bone ash determined by the

method described below. Of the ten poults started on the basal, only three survived; these had an average bone ash of 30.3%. Nine poults that received the sardine oil had an average bone ash of 45.1% while the eight poults that received the irradiated 7-dehydrocholesterol had an average bone ash of 49.0%. There was no mortality in the two groups of poults to which supplements were given.

These preliminary results indicated that the basal diet was satisfactory for producing rickets and that there might well exist distinct differences between different vitamins D in their ability to prevent rachitic manifestations in poults. Thus encouraged, a more extensive and quantitative comparison of four vitamin D supplements from different origins was undertaken. Two separate experiments were carried out in which these supplements were fed at various levels, experiment 2 being planned and carried out after the results of experiment 1 had been carefully studied.

The vitamin D supplements employed were: (1) U.S.P. reference cod liver oil no. 2; (2) a corn oil solution of an irradiated animal sterol; (3) a corn oil solution of irradiated 7-dehydrocholesterol; and (4) a fortified sardine oil.[2] These materials were chosen for the reason that they were representative of vitamins D of diverse origin and also of those largely used in modern poultry feeds. The reference cod liver oil was a standard test substance of known vitamin D content and in addition was presumed to be representative of cod liver oils in general. The fortified sardine oil was supplied with the assurance that all of its vitamin D activity came from fish oil sources. It was assumed that its vitamin D activity would be representative of sardine oil plus whatever high potency liver oil was used for increasing its D content. The irradiated animal sterol was prepared by irradiating with ultraviolet light the purified sterol mixture isolated from a species of mussel. The exact chemical nature of the vitamin D which it contained has not been reported. The irradiated 7-dehydrocholesterol, on the other hand, contained a single known vitamin D, namely D_3. Both irradiated products have come into wide commercial use under the designation of D-Activated Animal Sterol.

The various levels of the supplements were introduced into the diet by pre-mixing with the corn oil component. In order to establish their potency with accuracy the irradiated animal sterol, the irradiated 7-

[2] Thanks are expressed to E. Fullerton Cook, Chairman of the Committee of Revision of the Pharmacopoeia of the United States of America for the reference cod liver oil, to J. Waddell of E. I. du Pont de Nemours and Company for both the irradiated animal sterol and irradiated 7-dehydrocholesterol, and to T. D. Sanford of F. E. Booth Company for the fortified sardine oil.

dehydrocholesterol, and the fortified sardine oil were carefully assayed by the A.O.A.C. chick method in three separate laboratories. The results of the separate assays and the average value assigned to each supplement are summarized in table 2. It is believed that the assigned values have a high degree of accuracy.

White Holland and Bronze turkey poults were used in these investigations, representatives of both breeds being included in each group. Twelve to fourteen poults in each group were started when 1 day old and kept for 28 days in electrically heated battery brooders equipped with hardware cloth floors and automatic waterers. The brooders were

TABLE 2

A.O.A.C. chick assays of the vitamin D concentrates.

LABORATORY[1]	IRRADIATED ANIMAL STEROL	IRRADIATED 7-DEHYDROCHOLESTEROL	FORTIFIED SARDINE OIL
	Units per gm.	*Units per gm.*	*Units per gm.*
1	445 (4)[2]	340 (4)	435 (4)
2	404 (4)	342 (4)	408 (4)
	403 (3)		
3	423 (3)	415 (3)	450 (3)
	399 (3)	411 (3)	
Assigned values	415	375	430

[1] Laboratory 1, the Department of Agricultural and Biological Chemistry of The Pennsylvania State College. Laboratory 2, the Laboratory of Vitamin Technology, Chicago. Laboratory 3, the Biological Laboratory of E. I. du Pont de Nemours and Company, New Brunswick, N. J.

[2] The figure in parentheses following each assay value represents the number of groups of chicks used in that assay.

located in a laboratory from which all direct sunlight was excluded by covering the closed windows with heavy brown paper. The experimental diets and tap water were supplied ad libitum.

At the conclusion of each experiment the surviving poults were killed and the tibias removed for ash determination. This was done essentially as outlined in Official and Tentative Methods of Analysis ('40), except that the extraction was continued for 30 hours with 95% ethyl alcohol followed by 30 hours with ethyl ether. The extracted bones were dried to constant weight at 103°C. and then ashed by placing the crucibles in a cold furnace and raising the temperature slowly to 400°C. The total time required for this method of ashing approximated 16 hours, during the last three of which the temperature was increased to 800°C. While the bones were individually ashed only the

average value for each group has been recorded in the subsequent tables.

RESULTS AND DISCUSSION

In the first experiment nineteen groups of poults were used. These were divided as follows: poults in one group received the basal diet alone; six groups of poults received the basal plus levels of reference cod liver oil ranging from 20 to 200 units per 100 gm. of diet; and four groups of poults were placed on each of the other three supplements. The levels at which these supplements were fed were chosen in the hope that they would yield responses which could be compared quantitatively with those given by the reference oil. The range originally planned was from 20 to 120 A.O.A.C. units of each supplement per 100 gm. of diet but the final assigned A.O.A.C. potency, calculated after the turkey experiments had been started, modified these values slightly. Detailed information on the results obtained from experiment 1, such as mortality, body weight, dry weight of bone, and bone ash percentage is summarized in table 3.

TABLE 3

Summary of experiment 1.

GROUP	SUPPLEMENT PER 100 GM. BASAL DIET			MORTALITY DUE TO RICKETS %	RESULTS AT 4 WEEKS OF AGE			
	Source	mg.	A.O.A.C. units		No. of poults	Body weight gm.	Tibia weight gm.	Tibia ash %
1	None	0	0	92	1	112	.4221	25.5
2	Reference	174	20	92	1	192	.5780	32.4
3	cod liver	348	40	67	4	209	.6776	32.8
4	oil	696	80	0	12	260	.8352	35.0
5		1043	120	0	12	307	.9915	42.7
6		1391	160	0	12	285	.9199	42.2
7		1739	200	0	13	302	.9780	44.6
8	Irradiated	47	19	42	7	176	.5885	31.5
9	animal	93	39	9	10	247	.7770	37.4
10	sterol	186	77	0	13	265	.8069	47.7
11		279	116	0	12	276	.8703	48.7
12	Irradiated	47	17	64	4	186	.5845	31.0
13	7-dehydro-	93	35	8	11	217	.7302	34.8
14	cholesterol	186	70	0	13	335	1.0677	45.8
15		279	105	0	13	299	.9304	46.1
16	Fortified	44	19	69	4	187	.5648	27.4
17	sardine	89	38	42	7	210	.6933	31.3
18	oil	178	76	22	7	242	.7442	38.3
19		267	115	0	12	294	.9261	41.2

In order to confirm the surprising differences in efficacy noted among the supplements in the first experiment, a second was carried out. In this second experiment 20 groups of poults were used, these being distributed as in the first except that 7 groups of poults received reference cod liver oil. The levels fed of each supplement were adjusted somewhat from those used in the first experiment in order to facilitate quantitative comparisons. In addition, higher levels of reference cod liver oil were fed because of the evidence that 200 units did not produce maximum bone ash in the first experiment. Details and results of experiment 2 are presented in table 4.

TABLE 4

Summary of experiment 2.

GROUP	SUPPLEMENT PER 100 GM. BASAL DIET			MORTALITY DUE TO RICKETS %	RESULTS AT 4 WEEKS OF AGE			
	Source	mg.	A.O.A.C. units		No. of poults	Body weight gm.	Tibia weight gm.	Tibia ash %
1	None	0	0	88	1	137	.7106	28.3
2	Reference	348	40	10	9	226	.6112	33.5
3	cod liver	522	60	0	8	261	.7121	38.7
4	oil	696	80	0	8	295	.8676	40.5
5		870	100	0	10	284	.8411	42.6
6		1217	140	0	9	279	.8208	46.4
7		1739	200	0	10	302	.9825	47.1
8		2609	300	0	8	302	.9278	47.3
9	Irradiated	93	39	0	9	231	.6465	40.8
10	animal	140	58	0	8	264	.7394	46.2
11	sterol	186	77	0	9	237	.6829	49.1
12		233	97	0	5	287	.8478	49.1
13	Irradiated	93	35	0	7	203	.5869	39.1
14	7-dehydro-	140	52	0	8	234	.6563	42.5
15	cholesterol	186	70	0	7	282	.8747	47.6
16		233	87	0	7	252	.7505	49.1
17	Fortified	133	57	0	8	230	.6210	41.2
18	sardine	222	96	0	9	228	.6564	44.7
19	oil	311	134	0	8	283	.8650	47.5
20		444	191	0	9	326	.9388	49.8

The data representing the bone ash response obtained in experiments 1 and 2 are presented graphically in figures 1 and 2, respectively, the percentage bone ash being plotted against the number of A.O.A.C. units fed of each supplement.

From a study of the results of these experiments it was quite clear that the vitamins D in the two irradiated products were much more

effective in promoting calcification in turkey poults than those of the fish oils when compared on the basis of the A.O.A.C. chick units fed. The results of the two experiments agreed very well.

An inspection of the graphs indicates that the response curves of the two irradiated products have a different slope from the response curves of the fish oils. It is obvious, therefore, that in attempting to give numerical expression to the differences in efficacy under these conditions a different value will be obtained for every level of response at

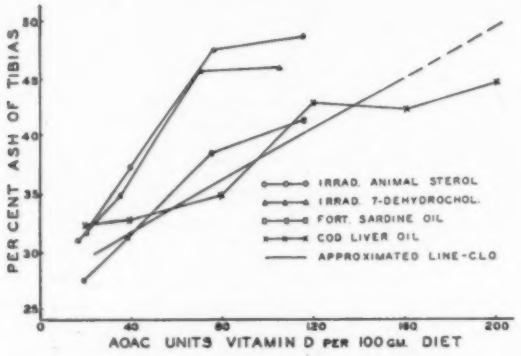

Fig. 1 Bone ash responses of poults fed vitamin D from different sources. Experiment 1.

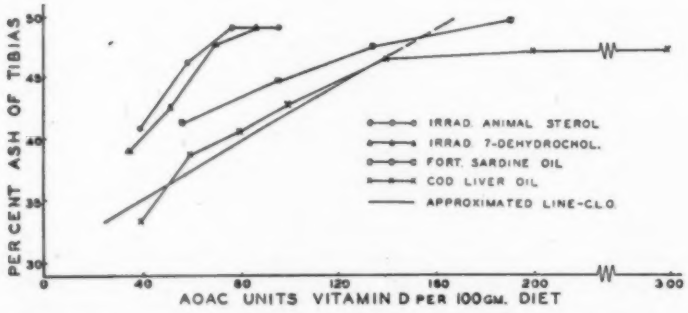

Fig. 2 Bone ash responses of poults fed vitamin D from different sources. Experiment 2.

which the comparison is made. For the present purpose a straight line of best fit has been drawn (by inspection) through the cod liver oil responses in each experiment. By placing on these approximated lines the equivalent bone ash responses of the other supplements the corresponding units have been read for each level fed. By comparing these "response" units with the number of A.O.A.C. units actually fed the

average efficacy ratio of each supplement as compared with the reference oil was calculated. These calculations for both experiments have been summarized and averaged in table 5.

TABLE 5

Efficacy ratio of the three supplements compared with reference cod liver oil.

SUPPLEMENT	GROUP NO.	TIBIA ASH %	A.O.A.C. UNITS FED	A.O.A.C. UNITS FROM C.L.O. LINE	EFFICACY RATIO CLO = 1
		Experiment 1			
Irradiated	8	31.5	19	38	2.0
animal	9	37.4	39	91	2.3
sterol	10	47.7	77	183	2.4 [1]
	11	48.7	116	192	1.7 [1]
					Average 2.1
Irradiated	12	31.0	17	33	1.9
7-dyhydro-	13	34.8	35	68	1.9
cholesterol	14	45.8	70	160	2.3 [1]
	15	46.1	105	170	1.6 [1]
					Average 1.9
Fortified	16	27.4	19	..	..
sardine	17	31.3	38	35	0.9
oil	18	38.3	76	99	1.3
	19	41.2	115	125	1.1
					Average 1.1
		Experiment 2			
Irradiated	9	40.8	39	87	2.2
animal	10	46.2	58	134	2.3
sterol	11	49.1	77	158	2.1
	12	49.1	97	158	1.6 [1]
					Average 2.1
Irradiated	13	39.1	35	73	2.1
7-dehydro-	14	42.5	52	106	2.0
cholesterol	15	47.6	70	145	2.1 [1]
	16	49.1	87	158	1.8 [1]
					Average 2.0
Fortified	17	41.2	57	91	1.6
sardine	18	44.7	96	120	1.3
oil	19	47.5	134	145	1.1 [1]
	20	49.8	191	164	0.9 [1]
					Average 1.2

[1] Value obtained by extending approximated straight line for the cod liver oil.

By this procedure it will be seen that the efficacy ratio for the irradiated animal sterol averaged 2.1 in both experiments, that of the irradiated 7-dehydrocholesterol 1.9 and 2.0, and that of the fortified sardine

oil 1.1 and 1.2. Obviously, for the reasons already mentioned, the ratios calculated at the high bone ash responses are relatively less valuable from a quantitative standpoint since it was only by extrapolating the straight line of the cod liver oil beyond the actual values given by this supplement that comparisons could be made at these levels. Actually, the reference oil, even when fed at 300 units per 100 gm. of diet never gave as high bone ash values as the other supplements. Consequently, if one compares the efficacy of the supplements at the level required to give maximum calcification, values appreciably greater than the averages given in table 5 would be indicated.

This suggests that turkeys, at least under the conditions of these experiments, utilize certain forms of vitamin D with degrees of efficacy that differ from chickens and exhibit a higher degree of species specificity than chickens. Certainly, it can be said that the A.O.A.C. chick unitage is not necessarily a true measure of the value of a vitamin D source for turkeys.

The results here reported confirm those of Jukes and Sanford ('39) in regard to the relative efficacy of reference cod liver oil and fortified sardine oil. It is to be remembered, however, that Jukes and Sanford ('39) used U.S.P. reference cod liver oil no. 1. While reference cod liver oil is measurably less effective than fortified sardine oil, on the basis of chick unit content, the slopes of their response curves are similar whereas both are distinctly different from the slopes of the irradiated products curves. If reference cod liver oil and fortified sardine oil can be assumed to be representative of fish oils generally, then 200 units per 100 gm. of diet from such sources may be considered an approximation of the "requirement" of turkey poults. In contrast, approximately 80 units from irradiated animal sterol or irradiated 7-dehydrocholesterol would satisfy the "requirement".

This interpretation may explain the results of Carver and Rhian ('42). Possibly the reinforced cod liver oil used by them contained an irradiation product since concentrates of irradiated animal provitamins are used in preparing such blends. It is, of course, also a possibility that fish oils from different species or from the same species at different times may vary greatly in the efficacy for turkeys of their vitamins D.

It is more difficult to explain Hammond's ('41) results with reference cod liver oil. Since his paper was submitted for publication in August, 1940, it may be assumed that he used reference cod liver oil no. 1, the same that was used by Jukes and Sanford ('39), yet he reported good protection with a level of 80 units per 100 gm. of diet. It is well known that the calcium and phosphorus contents of the diet in-

fluence the calcification of the bones but this would not seem to resolve the difficulty since Hammond's diet, while containing more calcium and phosphorus than the diet used in these experiments, contained less of both than the diet used by Jukes and Sanford. The question arises if, by chance, the "vitamin D-free commercial concentrate" of vitamin A used by Hammond may actually have carried some vitamin D. Unfortunately, there was no group of poults in his experiment that received the basal diet without the addition of a vitamin D concentrate.

SUMMARY

1. Different levels of vitamin D from four different sources, namely, U.S.P. reference cod liver oil no. 2, a sardine oil fortified with fish liver oils, an irradiated animal sterol, and irradiated 7-dehydrocholesterol were fed to poults as supplements to a rickets-producing diet during the first 4 weeks of life. All supplements were fed on the basis of their A.O.A.C. chick unit potency.

2. There were distinct differences in efficacy among the supplements, suggesting that the poult utilizes certain forms of vitamin D with degrees of efficacy that differ from the chick and in this response exhibits a higher degree of species specificity than the chick.

3. The vitamin D of the two irradiated products was distinctly more efficacious, on the basis of chick units fed, in increasing the ash content of the bones than the vitamin D of reference cod liver oil. Comparing the results obtained over the entire range of bone ash responses, this difference in efficacy is of the order of 2 to 1. While the fortified sardine oil was measurably more efficacious than the reference cod liver oil, it gave a response curve more nearly like that of cod liver oil than like the irradiated products.

4. It is concluded that the vitamin D potency expressed in A.O.A.C. chick units is not necessarily a true measure of the value of a vitamin D supplement for turkeys.

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THE PYRIDOXINE CONTENT OF FRESH, PASTEURIZED, EVAPORATED, AND DRIED MILK[1]

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There are only a limited number of reports in the literature concerned with the pyridoxine content of foods. Perhaps this is caused by the fact that the place of pyridoxine in human nutrition is still not clearly defined or by the fact that the available methods of assay have been time-consuming and often inaccurate. Teply, Strong and Elvehjem ('42) have investigated the pyridoxine content of wheat and recently both Atkin and associates ('43) and Stokes and coworkers ('43) have presented several values for cereals and cereal products. Henderson, Waisman and Elvehjem ('41) have reported values for the pyridoxine content of meat. Some of the papers mentioned have also included one or more values for the pyridoxine content of fresh milk. However, the only values on processed milk are given in the paper by Atkin et al. ('43) and since the value given for evaporated milk is nearly 50% lower on a reconstituted basis than the values for fresh milk given in the same paper, a processing loss is suggested. Since a loss of this magnitude is not consistent with our knowledge of the stability of pyridoxine toward heat and oxidation, an investigation of the pyridoxine content of fresh and processed milk has been undertaken.

The microbiological method of Stokes and associates ('43) using the "pyridoxinless" mutant, no. 299 of Neurospora Sitophila was chosen for the investigation. The organism does not respond to pseudopyridoxine and gives reasonable agreement with rat assays on many materials. Furthermore, the method does not require any special apparatus and is sufficiently simple so that with a large autoclave and an incubating room ten to sixteen samples can be assayed simultaneously.

[1] The author wishes to thank the officials of the Pet Milk Company for releasing this report for publication and especially Dr. E. A. Louder, Technical Director, for his part in releasing the report as well as his supervision of the research program. He also wishes to acknowledge the assistance of Mr. H. E. O. Heineman in reviewing the manuscript, and the kindness of Dr. J. T. Stokes, Merck & Co., for supplying the culture of the organism used.

In the selection of samples for assay it was not possible to obtain entirely comparable samples of the various kinds of milk. The fresh samples were pooled samples from several farms. The pasteurized milk was from two plants, one small and one fairly large plant. The evaporated milk samples were from several plants scattered through California, Idaho, Illinois, Kansas, Kentucky, Missouri, Ohio, Virginia, and Wisconsin. Because of the wide geographic distribution of the plants and because each can of evaporated milk is an extremely accurate sample of the pooled processed milk from many farms, the values for evaporated milk are probably fairly representative of the pyridoxine content of milk throughout the United States as determined by the neurospora method. All evaporated samples were irradiated and contained 135 I. U. of vitamin D per reconstituted quart. The dry skim

TABLE 1

Pyridoxine content of fresh and processed milk samples

PRODUCT	NUMBER OF SAMPLES ASSAYED	MG. PYRIDOXINE[1] PER LITER FRESH OR RECONSTITUTED MILK		AVE. MG. PYRIDOXINE[1] ON UNDILUTED BASIS PER LITER
		Average	Range	
Fresh milk	10	0.67	0.48–0.95	0.67
Pasteurized milk	10	0.65	0.47–0.90	0.65
Irradiated evaporated milk	22	0.73	0.51–1.05	1.46
				PER KILOGRAM
Dry skim milk	10	0.66	0.31–1.14	6.8
Dry whole milk	6	0.65	0.40–1.12	5.0
Average (unweighted)		0.67		

[1] Pyridoxine is reported as such. To convert to pyridoxine hydrochloride divide by 0.82.

milks were from four plants in Michigan, Utah, and Wisconsin. The dry whole milks were from a single plant. All the samples were collected in the fall, and most of them during October and November when fall pastures were still available throughout much but not all the areas from which samples were drawn. Assays were started on the processed milks within a few weeks after preparation.

The results of the investigation are recorded in table 1. The agreement between the average values on the various types of milk is very good. This indicates that there are no significant losses of pyridoxine in the preparation of the pasteurized, irradiated evaporated or dry milk. Because the samples are not entirely comparable and because the method of assay is not highly precise, it is impossible to say whether small losses may or may not occur.

Most of the reports found in the literature record only a few values for milk and these fall within the range of values in table 1. The values obtained by Atkin et al. ('43) with their yeast method, while within this range, are somewhat lower, especially since their values are in terms of pyridoxine hydrochloride rather than pyridoxine. Whether the difference between the values is merely normal variation or difference in the methods of assay is not clear. The one value obtained by rat assay and reported by Henderson, Waisman and Elvehjem ('41) is higher but when converted to a pyridoxine basis the value, 1.1 μg per milliliter is within the upper limits of the range reported in table 1. Stokes and coworkers ('43) assayed two samples of milk by the neurospora method and found values of 0.58 μg. per milliliter for each. This value is in favorable agreement with the average reported in table 1.

The reason for the wide range of the values in table 1 is not clearly understood but can probably be largely assigned to normal variation. However, we must also consider that the method of assay used is not highly precise. Stokes, Larsen, Woodward, and Foster ('43) suggest an experimental error of 15% for the neurospora method. When the method is applied to milk in our laboratory the results are somewhat more variable. When pyridoxine was added to twelve samples of fresh, evaporated, and dry whole milk and carried through the entire procedure an average total recovery of 86% was obtained. The values ranged between 80 and 93% with the single exception of one 72% recovery. The reason for these low recoveries is not apparent. A single recovery on a non-milk product (corn germ) was excellent.

Milk has often been classed as a food of low pyridoxine content. This is true when it is compared on a fresh or fluid basis with fresh meats or with air dry cereals. When compared on a dry matter basis, the pyridoxine content of milk falls in the same range as the values for cereals given by Stokes et al. ('43) and by Teply and coworkers ('42). On the same basis milk is about ½ to ¼ as rich in pyridoxine as most meat according to the data given by Henderson, Waisman and Elvehjem ('41).

SUMMARY

1. The pyridoxine content of cow's milk has been investigated and an average value of 0.67 mg. per liter has been found for fifty-eight samples.

2. There were no significant losses of pyridoxine in the preparation of the pasteurized evaporated or dried milks assayed.

3. On a dry basis milk compares favorably with cereals in pyridoxine content and contains one-half to one-fourth as much as most meats.

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THE RETENTION OF VITAMINS IN PORK HAMS DURING CURING [1]

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The retention of thiamine, riboflavin and nicotinic acid during storage, curing and cooking of pork hams was reported in a recent paper by Schweigert et al. ('43 a). In this work the percentage retention of the vitamins during curing was calculated on the basis of the residual solids content (obtained by subtracting the moisture and fat percentage from 100) of the meat before and after treatment. Shortly after publication, Rice [2] suggested that the figures given for the vitamin retention during curing might be low due to differences in salt content of the fresh and cured hams. The protein content of the ham samples used in the previous experiments was determined and the per cent of protein in the fresh and cured hams was 14.5% and 16.0%, respectively. The residual solids content of the fresh and cured hams was 15.1% and 18.9%, respectively, Schweigert et al. ('43 a). The difference between the per cent of residual solids and protein, therefore, was 0.6% in the fresh ham and 2.9% in the cured ham. If this difference was due entirely to salt, then an error was present in our results. In order to check this difference the per cent retention was calculated on the basis of the vitamin content per gram of protein in the fresh and cured hams.

To repeat this experiment four additional pairs of hams were studied. These hams were analyzed for their vitamin content, and the vitamin retention was calculated on the basis of original weight of the fresh ham and the final weight of the cured ham. For comparison the vitamin retention was also calculated on the basis of the vitamin content per gram of protein in the fresh and cured hams.

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[2] Personal communication from E. E. Rice, Swift and Company. We are deeply indebted to Dr. Rice for calling this possible error to our attention.

EXPERIMENTAL

Four hog carcasses of approximately the same size were selected for the study. The right and left hams from each carcass were removed and trimmed similarly. They were weighed immediately and the right and left hams were alternated for curing and for fresh samples. The hams used in the curing experiment were cured commercially. The hams were first injected with pickling solution equivalent to 8% of their original weight and placed in a pickling solution for 13 days. They were removed from the pickle and placed in a smoke house until

TABLE 1

Vitamin retention during curing of pork hams calculated on basis of fresh and cured weights.

HAM SAMPLE	HOG NO. 1		HOG NO. 2		HOG NO. 3		HOG. NO. 4	
	fresh	cured	fresh	cured	fresh	cured	fresh	cured
Weight (gm.)	5655	5535	6065	5945	5260	5045	5880	5765
Thiamine in meat (μg./gm.)	7.68	6.04	7.05	5.62	6.06	5.12	9.00	7.50
Thiamine in meat (total mg.)	43.5	33.4	42.8	33.4	31.8	25.8	52.8	43.2
Per cent thiamine retained	77		78		81		82	
Riboflavin in meat (μg./gm.)	2.13	1.99	1.98	2.07	2.43	2.43	2.02	2.05
Riboflavin in meat (total mg.)	12.0	11.0	12.0	12.3	12.8	12.3	11.9	11.8
Per cent riboflavin retained	92		102		96		99	
Nicotinic acid in meat (μg./gm.)	27.7	25.3	15.0	15.3	26.3	22.3	16.7	22.0
Nicotinic acid in meat (total mg.)	157	143	91	91	138	113	99	127
Per cent nicotinic acid retained	91		100		82		128	

the internal temperature of the ham reached 148° F. After curing the final weight of the cured ham was about 96% of the original weight of the fresh ham.

The fresh and cured hams were prepared for analysis as described by Schweigert et al. ('43 a). The protein nitrogen was determined by the Kjeldahl method, and the per cent of protein was calculated by multiplying the per cent of nitrogen by 6.25. The thiamine content of the samples was determined by the method of Hennessy ('42) with

TABLE 2

Vitamin retention during curing of pork hams calculated on the basis of protein content.

HAM SAMPLE	HOG NO. 1		HOG NO. 2		HOG NO. 3		HOG. NO. 4	
	fresh	cured	fresh	cured	fresh	cured	fresh	cured
Per cent protein	16.25	15.60	13.0	12.6	16.0	15.1	14.8	14.6
Thiamine (μg./gm. meat)	7.68	6.04	7.05	5.62	6.06	5.12	9.00	7.50
Thiamine (μg./gm. protein)	47.2	38.8	54.2	44.8	37.9	33.9	60.8	51.4
Per cent thiamine retained	82		83		89		85	
Riboflavin (μg./gm. meat)	2.13	1.99	1.98	2.07	2.43	2.43	2.02	2.05
Riboflavin (μg./gm. protein)	13.1	12.8	15.2	16.5	15.2	16.1	13.6	14.0
Per cent riboflavin retained	98		108		106		102	
Nicotinic acid (μg./gm. meat)	27.7	25.3	15.0	15.3	26.3	22.3	16.7	22.0
Nicotinic acid (μg./gm. protein)	170	163	115	122	164	148	113	149
Per cent nicotinic acid retained	96		106		90		132	

modifications by McIntire et al. ('43), the nicotinic acid by the method of Snell and Wright ('41), with modifications by Krehl et al. ('43), and riboflavin by the fluorometric method of Conner and Straub ('41) with modifications described by Andrews ('43).

All of the vitamin analyses were made directly on the samples without drying. The per cent of vitamin retention was calculated in two ways: (1) by dividing the total milligrams of the vitamin in the cured meat by the total milligrams of the vitamin in the fresh meat (table 1); (2) by dividing the vitamin content per gram of protein in the cured meat by the vitamin content per gram of protein in the fresh meat (table 2).

DISCUSSION

When the vitamin retention in the cured hams which were used in the previous experimental work was calculated on the basis of vitamin content per gram of protein, the per cent retentions were 84% for thiamine, 96% for nicotinic acid and 104% for riboflavin. These figures for vitamin retentions are higher than the retentions, 73% for thiamine, 84% for nicotinic acid and 92% for riboflavin, which we reported when the vitamin retention was calculated on the basis of residual solids. The original and final weights were not available for these hams, consequently a comparison of retentions could not be made on the basis of weight change during curing.

The riboflavin contents of the fresh hams, 1.98–2.43 μg. per gram of fresh ham, are of the same magnitude as previously reported by McIntire et al. ('43), Schweigert et al. ('43a), and Cheldelin and Williams ('42). The thiamine values ranged from 6.06 to 9.0 μg. per gram of fresh ham. This range is low compared to the range of thiamine values for fresh hams and loins, 7.4 to 15.2 reported by McIntire et al. ('43), and 9.8 μg. per gram reported by Cheldelin and Williams ('42). The nicotinic acid values ranged from 15.0 to 27.7 μg. per gram of fresh ham which are also lower than most hams previously analyzed. These values emphasize the rather wide variations in vitamin content per gram of fresh tissues which have been observed by Rice et al. ('43), McIntire et al. ('43a) and Schweigert et al. '43a). The nutritional history of the animal may play an important role in causing variations in vitamin contents of animal tissues (Schultz et al., '39; Schweigert et al., '43 b; and Miller et al., '43), as well as variations in fat content, age of the animal and other factors.

The per cent of vitamin retentions during curing of pork hams when calculated on the basis of net weight (table 1) averaged 80% for thiamine, 97% for riboflavin and 100% for nicotinic acid. The nicotinic

acid retention of 128% observed in hog no. 4 is obviously due to an error of some kind. A complete repetition of the analysis showed that the error was not due to the nicotinic acid determination. The most probable explanation is variation in the nicotinic acid content in this particular pair of hams although the thiamine and riboflavin retentions in this pair of hams closely parallel the retentions which were obtained in other hams. The per cent of retention when calculated on the basis of the vitamin content per gram of protein averaged 85% for thiamine, 104% for riboflavin and 106% for nicotinic acid. The cured hams are all slightly lower in protein than the fresh hams which may be due to small losses of soluble nitrogenous materials during the curing of the hams.

The best method of calculating the per cent of vitamin retention seems to be on the basis of the original and final weights of the samples tested. It is the most direct method since it does not involve additional determinations and calculations which is the case when the protein content is used as the basis for calculation.

SUMMARY

1. The thiamine, riboflavin and nicotinic acid retentions during curing of pork hams have been studied using two methods of calculation.

2. When calculated on the basis of the total vitamin content of the fresh and cured hams, 80% of the thiamine, 97% of the riboflavin and 100% of the nicotinic acid were retained.

3. When calculated on the basis of the vitamin content per gram of protein in the fresh and cured hams, 85% of the thiamine, 104% of the riboflavin and 106% of the nicotinic acid were retained.

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STUDIES OF PANTOTHENIC ACID DEFICIENCY IN DOGS

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THREE FIGURES

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In the course of studies of pantothenic acid deficiency in dogs, it was found that pups survived depletion approximately 1 month, whereas adults could survive 6 months or longer [1] (Seeler and Silber, unpublished). Schaefer, McKibbin, and Elvehjem ('42) reported that growing pups have a greater requirement for pantothenic acid than adult dogs, and Unna and Richards ('42) observed a decrease in requirement with increase in age in rats. In the above dog studies it was impossible to ascertain the degree of pantothenic acid deficiency prior to the stage of collapse. Further studies therefore seemed indicated, with attempts to correlate excretion and tissue levels of pantothenic acid with other signs which might permit a more accurate determination of the onset of the deficiency.

EXPERIMENTAL

A total of twenty-two dogs were used. These included three litters of pups and one litter of young dogs. The basal diet consisted of vitamin-free casein (30%), dextrose (41%), hydrogenated vegetable fat (21%), corn oil (0.15%), cod liver oil (2%), Osborne-Mendel salt mixture (3%), and bone ash (2.85%). All dogs received 50 mg. alpha tocopherol every 2 weeks orally. All dogs also received the following supplement daily added to the first portion of the diet consumed in the morning: thiamine, riboflavin and pyridoxine, 1 mg. each; niacin, 20 mg.; choline chloride and inositol, 200 mg. each. In addition to the above supplement one group of control dogs received 40 mg. calcium pantothenate and 5 gm. dried beef liver [2] daily, regardless of body weight. Another group received the calcium pantothenate but no dried beef liver.

[1] One adult dog has been receiving the deficient diet since July, 1940, and is still maintaining its weight (November, 1943).

[2] The liver contained approximately 200 μg. pantothenic acid, 80 μg. riboflavin, 6.5 μg. thiamine, 25 μg. biotin, and 30 μg. folic acid per gram.

Prior to depletion all dogs were given vermifuge and fed the basal diet plus all the supplements including calcium pantothenate and dried beef liver for 2 weeks; that is, the pups were given the supplemented basal diet from the age of 6 weeks to 8 weeks, and the adults received the same ration from the age of 6 to 6½ months in order to accustom them to the diet and to insure saturation with pantothenic acid at the beginning of the experiment.

At the end of this 2-week equilibration period, the calcium pantothenate and the liver were omitted from the supplement fed to five pups and two adults, and the liver alone was omitted from the supplement given to seven other pups and two adults. The six remaining pups continued to receive both calcium pantothenate and liver throughout the experiment (table 1). Of these fifteen control dogs, only five were

TABLE 1

Dietary regimen and weights of depleted dogs and controls.
(CaPa = calcium pantothenate)

DOG NO.	LITTER NUMBER	BASAL DIET	ADDITIONS TO THE SUPPLEMENT	INITIAL WEIGHT IN KILO.	TERMINAL WEIGHT IN KILO.	TERMINAL AGE IN MONTHS
206 M	1	Ad libitum	None	2.5	6.4 [1]	5
210 F	1	Ad libitum	None	3.1	5.2	4
212 M	1	Ad libitum	None	3.1	4.1	4
213 M	2	Ad libitum	None	4.6	6.6 [1]	5
215 F	2	Ad libitum	None	3.4	8.2 [1]	5
201 M	3	Ad libitum	CaPa	2.4	10.0	5 [2]
202 F	3	Ad libitum	CaPa	2.7	8.8	5 [2]
203 M	3	Ad libitum	CaPa	3.3	10.1	5 [2]
		Paired with				
207 F	1	206	CaPa	2.7	6.8	5
208 M	1	210	CaPa	2.7	5.2	4
216 F	2	213	CaPa	4.2	7.5	5
217 M	2	215	CaPa	3.7	9.3	5
211 F	1	206	CaPa and liver	1.9	6.1	5
209 M	1	210	CaPa and liver	3.5	6.3	4
218 M	2	213	CaPa and liver	4.1	8.8	5
214 F	2	215	CaPa and liver	3.2	8.9	5
204 M	3	202	CaPa and liver	1.7	8.1	5 [2]
205 F	3	203	CaPa and liver	3.7	10.3	5 [2]
63 M	4	Ad libitum	None	17.2	14.5	10
73 M	4	Ad libitum	None	14.8	15.4	9½
		Paired with				
65 M	4	63	CaPa	16.2	13.9	10
71 M	4	73	CaPa	13.7	14.3	9½

[1] Pups 206, 213 and 215 received CaPa orally after 56–70 days on depletion.

[2] Pups 201 to 205, inclusive, survived beyond 5 months. Four of them are still living after 2 years on the same diet.

allowed to eat ad libitum; the other ten were fed only as much as their paired litter mates on the "depletion diet" voluntarily consumed.

For the pantothenic acid determinations, 24-hour urine specimens were collected under toluene in amber bottles, blood samples were taken from the jugular vein after overnight fast, and fresh tissues were taken at autopsy. The microbiological assay of Pennington, Snell, and Williams ('40) as modified by Silber and Mushett ('42) was employed for the pantothenic acid determinations.

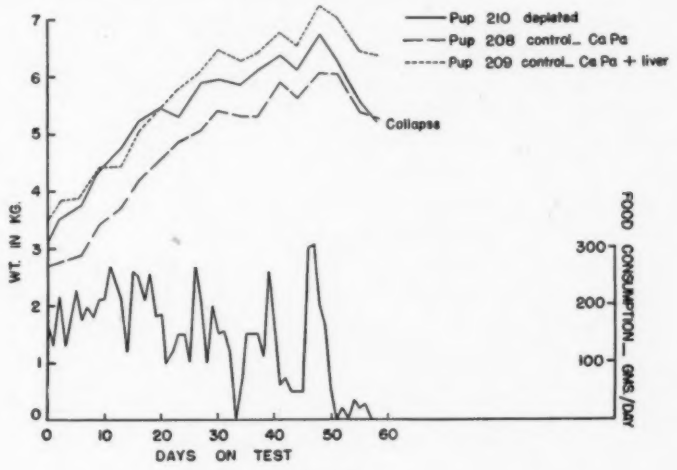

Figure 1

RESULTS

Within 2 to 3 weeks after withdrawal of calcium pantothenate, the pups showed an erratic food intake, whereas it required 7 to 8 weeks to produce a similar effect in the depleted adults. A decrease in food consumption was noted as the depletion progressed (figs. 1, 2). Two of the pups collapsed after 2 months and were sacrificed. At this time the other three in this group were given daily oral doses of 4 mg. calcium pantothenate per kilogram and showed simultaneous increases in food intake and growth (fig. 2). A similar response to oral therapy had been observed in two adult dogs in a previous study. Within a 10-week period these animals made weight gains of 57% and 113%, respectively.

After 7 to 8 weeks on the depletion diet, three of the pups became irritable and weak. One of the young adults evidenced a similar condition after 3 months. The irritability was particularly evident when the dogs were grasped at the flank. Spasticity was observed in the hind

legs during the last week on the depletion diet. Although the pups and adults on paired feeding, due to the restricted food intake, lost weight to about the same extent as the depleted dogs, they remained active and had voracious appetites. The ad libitum control pups maintained adequate food consumption and growth (fig. 3) and appeared to be normal.

Weekly examinations of the dogs revealed no evidence of gross changes in the mouth, eyes, ears, anus, or skin. The hair of the depleted dogs appeared somewhat coarser than that of the controls, with

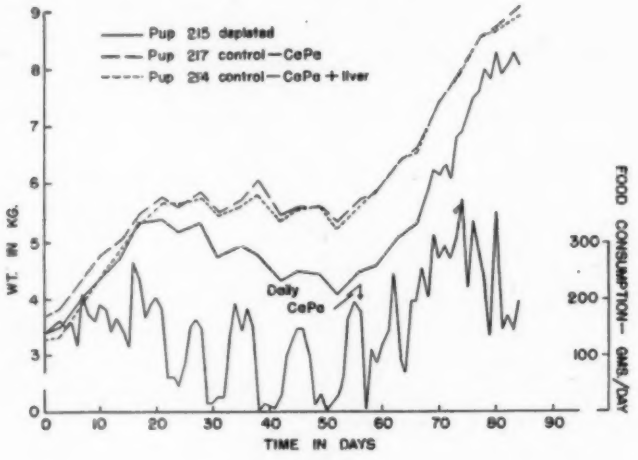

Figure 2

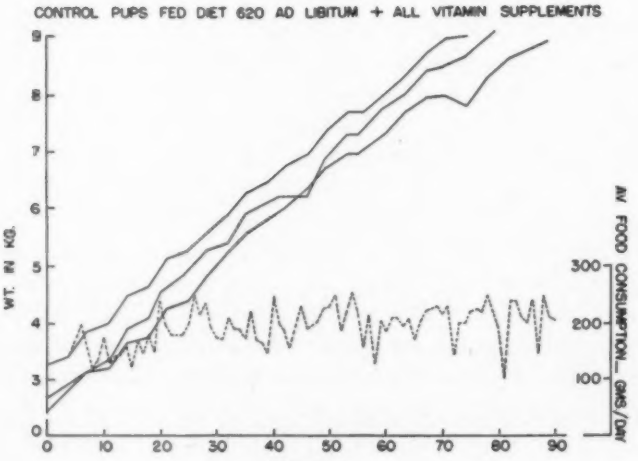

Figure 3

those receiving liver supplement possessing the smoothest coat, but there was no indication of alopecia, graying, or other skin disorder. Diarrhea was rarely observed in pups receiving liver and only occasionally in all other pups except two on depletion diet which had a loose or watery stool almost every day.

At the time of collapse, the corneal reflex was sluggish, the mouth was open with excessive salivation, and when aroused the pups were extremely weak and irritable. They behaved as though they could not see well, stumbling into and snapping at stationary objects.

After 52–54 days on test, the depleted pups and their controls showed no indication of liver damage when the bromsulphalein dye retention test was used. The older dogs also showed no evidence of dye retention when tested after 11–12 weeks on experiment.[3] In spite of these negative results, some of the pups were found upon autopsy to have fatty livers.

Samples of gastric juice were taken from fasting pups ½ hour after subcutaneous administration of 0.1 mg. histamine per kilogram (after 55 days on test). Four hundred to 1170 m. equiv. free acid and 450 to 1210 m. equiv. total acid per 100 ml. gastric contents were found, with no difference between depleted dogs and their controls.[3]

The total lipoid and cholesterol contents of the blood of the deficient pups and adults were investigated by Scudi and Hamlin ('42) and found to be lower than in the controls and increased after oral administration of calcium pantothenate.

Gross examination of the organs of the dogs revealed no differences between depleted dogs and their controls with the exception of the livers. The livers of depleted dogs and, to a lesser degree, those of the controls not receiving a liver supplement in their diets had a yellowish appearance, due to accumulation of fat. This was confirmed by microscopic examination of the tissue. Fatty livers were also described by Schaefer et al. ('42). The fatty livers were not caused by inanition because the paired feeding controls which received the 5 gm. dried liver supplement did not show this condition. Moreover, the finding of less severe fatty livers among the controls which were not fed liver indicates that an other factor (or factors) may be involved.

Excretion of pantothenic acid in urine and feces

The pantothenic acid excretion in the urine decreased strikingly the first day after withdrawal of calcium pantothenate (table 2). There-

[3] We are indebted to Dr. D. Tennent and Mr. S. Kuna for their assistance in making these tests.

after, excretion in the pups remained above normal for 3 weeks and then decreased until the animals collapsed. In the adults, which did not reach the state of collapse even after 13 weeks of depletion, the rate of pantothenic acid excretion remained relatively constant throughout this period and was in the range of nine dogs maintained on stock diet (0.04 mg. per day). That the feeding of large amounts of calcium pantothenate before giving the dogs the depletion diet was responsible for this sustained urinary excretion is indicated by the fact that two other dogs, not previously dosed, consistently excreted only 0.002 to 0.010 mg. per day after receiving the same deficient diet for 2 to 3 months. Furthermore, pups which were not given massive doses of

TABLE 2

Urinary excretion of pantothenic acid during depletion.
(Data expressed in milligrams per 24 hours after intervals indicated)

PUP NO.	BEFORE DEPLETION	1 DAY	1 WEEK	2 WEEKS	3 WEEKS	5 WEEKS	7 WEEKS	9 WEEKS
206	11.5	0.24	0.04	0.09	0.07	0.010	0.052	0.008
210	10.4	0.83	0.08	0.12	0.23	0.017	....	....
212	12.2	0.18	0.06	0.10	0.08	0.007	0.025	....
213	5.7	0.29	0.10	0.10	0.09	....	0.013	....
215	10.4	0.20	0.13	0.12	0.27	0.050	0.006	0.007
Average	10.0	0.35	0.10	0.13	0.15	0.021	0.024	0.008

ADULT NO.	BEFORE DEPLETION	1 DAY	2 WEEKS	3½ WEEKS	5½ WEEKS	7½ WEEKS	9½ WEEKS	13 WEEKS
63	0.5	0.20	0.050	0.040	0.014	0.040	0.050	0.055
73	3.3	0.12	0.080	0.035	0.026	0.022	0.070	0.033
Average	1.9	0.16	0.065	0.038	0.020	0.031	0.060	0.045

calcium pantothenate prior to receiving the deficient diet, have been found to collapse after only 1 month. This also indicates that the calcium pantothenate dosage had a prolonged effect, which not only maintained excretion levels but even permitted appreciable growth during the first 3 weeks of depletion (figs. 1, 2).

The paired-feeding control pups, which continued to receive 40 mg. calcium pantothenate daily, excreted an average of 14.0 mg. urinary pantothenic acid per day. The adults excreted an average of 0.7 mg. per day. Aqueous extracts of 24-hour fecal specimens from depleted, treated (40 mg. calcium pantothenate per day), and stock diet dogs assayed, respectively, 0.05–0.5 mg. per day, 10.0–20.0 mg. per day, and 1.3–2.8 mg. per day. The fecal weight from depleted dogs, however,

was only 5–30 gm. per day, whereas stock diet dogs excreted 200–600 gm. in the same period, so there is little difference between these two groups when the comparison is made on the basis of fecal pantothenic acid concentration. The fecal pantothenic acid assay appears to be valid because baryta treatment quantitatively destroyed the pantothenic acid content of such extracts.

TABLE 3

Pantothenic acid content of tissues of dosed, depleted, and normal dogs. Data expressed in micrograms per gram tissue (wet weight)

TISSUE	PUPS DEPLETED 210, 212	CONTROL PUPS 207, 208, 209 211	CURED PUPS 206, 213, 215	DEPLETED YOUNG ADULTS 63, 73	CONTROL YOUNG ADULTS 65, 71	THREE STOCK DOGS [1]
Liver	25	73 (2)	65	35	55	62
Kidney	42	68	47	49	63	47
Brain	16	43	30	24	41	22
Heart muscle	18	31	35 (2)	23	50	20
Pancreas	24	30	18 (2)	28	22	20
Leg muscle	5	23	22	7	26	11
Adrenal	14 (1)	28	23 (2)	24	38	23 (2)
Spleen	7	10	9	9	7	8
Lung	5	7	8	12	8	6
Blood	0.1 (4)	0.4 (13)	0.3 (2)	0.1	0.3	0.2 (12)

Numbers in parentheses indicate number of cases included in the average value given, wherever it differs from the number of animals listed.

Tissue levels of pantothenic acid

Nine or ten tissues from each of sixteen dogs were assayed for pantothenic acid as shown in table 3. With few exceptions, the tissue levels of the depleted animals were lower than those of the controls. The fact that the young adults were not as severely depleted as the pups was borne out by the less pronounced differences in tissue levels. When the tissue levels of the experimental dogs are compared with those of dogs on stock diet, two points are evident: (1) the control dogs which had received 40 mg. calcium pantothenate daily had more pantothenic acid in their blood, kidney, brain, and muscle tissues than the stock diet dogs which had an intake of 1.5–3.0 mg. dietary pantothenic acid per day, and (2) the depleted dogs had lower than normal levels only in liver, muscle, brain and blood.

DISCUSSION

Although significant decreases have been found in the pantothenic acid levels of blood, liver, muscle, brain, urine, and feces of severely

depleted dogs, the possibility of revealing a deficiency state by any single determination seems slight. The differences between the levels of stock diet dogs and depleted dogs are not great enough to exclude biological variation and experimental error as complicating factors.

The pathological findings of Schaefer et al. ('42) that pantothenic acid depleted pups developed a mottled thymus and hemorrhagic degeneration of the kidney have not been observed in our experiments. It should be noted that our diets and vitamin supplements differed in several respects from theirs.

The fact that adult dogs can survive depletion much longer than pups might be the result of (1) a lower requirement for maintenance than for growth, (2) ability to utilize significant amounts of pantothenic acid already in the tissues, and (3) intestinal synthesis.

When calcium pantothenate was administered before a depletion period was started, the blood and urinary pantothenic acid levels as well as growth remained essentially normal during the first 3 weeks. When, on the other hand, depletion was started without previous calcium pantothenate dosage, pups collapsed after 1 month and adults excreted only 0.002–0.010 mg. per day after only 2 to 3 months. A prolonged effect of massive dosage is indicated.

SUMMARY

1. The first manifestations of pantothenic acid deficiency in pups were an erratic, decreasing appetite and a decrease in urinary excretion of the vitamin. Growth and food intake showed parallel decreases prior to collapse after 2 months. The deficiency responded to oral therapy.

2. Older dogs showed a delayed effect on appetite; the urinary pantothenic acid content was decreased, but was still essentially normal after 3 months depletion. This is in agreement with the fact that the pantothenic acid requirement of older dogs is lower.

3. The concentration of pantothenic acid in blood and other tissues of pantothenic acid depleted dogs was below that of controls dosed with large amounts of calcium pantothenate; however, when compared with stock diet dogs, the only significantly low pantothenic acid levels were found in liver, muscle, brain and blood. Repeated oral dosage with calcium pantothenate increased tissue levels above those found in dogs on stock diet and appeared to have a delaying effect on the progress of subsequent depletion.

4. The only gross pathological changes observed as a result of the deficiency were fatty livers. These were observed primarily in the

depleted pups but also, to a lesser degree, in the control dogs on the same diet supplemented with calcium pantothenate. This was not true of control dogs which received a dried beef liver supplement in addition. Spasticity was observed in the hind quarters during the last week of the deficiency.

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ERRATUM

Berryman, George H., and Paul E. Howe

A short method of calculating the nutritive value of diets, concluded

J. Nutrition, vol. 27, no. 3, March, 1944

Correct as follows:

TABLE 4.

1. "Tomatoes" instead of "Totatoes".
2. Value for fat per lb. of citrus fruits should be "1" gm. instead of "47".
3. Value for Vitamin A per lb. of citrus fruits changed from 96 to 380 per lb. (IU.)

TABLE 5.

1. Value for nicotinic acid per lb. of vegs. leafy green and yellow changed from .27 mg. to 2.7.
2. Value for Vitamin A per lb. of citrus fruits changed from 90 to 360 (IU.)